DESIGN AND SYNTHESIS OF NEW ANTICANCER AGENTS

THESIS SUBMITTED TO BUNDELKHAND UNIVERSITY FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

PHARMACY

BY

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CERTIFICATE

This is to certify that the thesis entitled "**Design and Synthesis of New Anticancer Agents**" submitted to the Bundelkhand University, Jhansi (U.P.), in fulfillment of requirement for the award of the degree of **Doctor of Philosophy in Pharmacy,** embodies the original research work carried out by **Mr. Vivek Kumar** (Enrollment No. BU/2004/5099) under our supervision. This work has not been submitted in part or full for the award of degree in this or any other University. That the candidate has put in an attendance of more than 200 days with us.

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I hereby declare that the thesis entitled "**Design and Synthesis of New Anticancer Agents**" embodies the results of the original research work carried out by me at the Institute of Pharmacy, Bundelkhand University, Jhansi and Division of Medicinal Chemistry, Chemical Research, Dabur Research Foundation (DRF), 22, Site IV, Sahibabad, Ghaziabad (U.P.). This work has not been submitted in part or full for the award of degree in this or any other University.

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TO WHOM IT MAY CONCERN

This is to certify that **Mr.Vivek Kumar** has carried out his project work on "Design and Synthesis of New Anticancer Agents" for the award of Ph.D. degree in the subject of Pharmaceutical chemistry/ Medicinal Chemistry in Chemical Research Laboratory.

He has performed his task with sincerity and dedication and submitted a relevant project report on the above topic.

We wish him all success for a bright future.

for Dabur Research Foundation

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Vivek Kumar

Vivek Kumar

DEDICATED

TO

THE ALMIGHTY

AND

MY RESPECTED PARENTS

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CHAPTER-1

INTRODUCTION

1. INTRODUCTION

1.1. General Introduction:

Cancer is not just one disease, but a large group of almost one hundred diseases. Its two main characteristics are uncontrolled growth of the cells in the human body and the ability of these cells to migrate from the original site and spread to distant sites. If the spread is not controlled, cancer can result in death.

The word 'cancer' derives from the appearance of solid tumors as noted on post-mortem examination by early physicians, who likened their appearance to that of a crab (Cancer) because of the irregular and disorganized appearance of the threads of the tumor radiating from a central body.

Cancer can attack anyone. Since the occurrence of cancer increases as individual's age increases, most of the cases are seen in adults, middle-aged or older. Sixty percent of all cancers are diagnosed in people who are older than 65 years of age. The most common cancers are skin cancer, lung cancer, colon cancer, breast cancer (in women), and prostate cancer (in men). In addition, cancer of the kidneys, ovaries, uterus, pancreas, bladder, rectum, and blood and lymph node cancer (leukemia and lymphoma) are also included among the 12 major cancers that affect most individuals.

Tumors are of two types, benign or malignant. A benign tumor is not considered cancer. It is slow growing, does not spread or invade surrounding tissue, and once it is removed, it doesn't usually recur. A malignant tumor, on the other hand, is cancer. It invades surrounding tissue and spreads to other parts of the body. If the cancer cells have spread to the surrounding tissues, then, even after the malignant tumor is removed, it generally recurs.[1]

1.2. Cancer Treatments:

Despite the major achievements in different new areas of drug discovery research, the successful treatment of the cancer still remains a significant challenge. The first drug, N-mustard appeared for more than five decades ago. Afterwards, a number of DNA alkylating agents, antimetabolites and animitotic agents discovered and several of them are still in use for treatment of cancer.[2]

Taxol, a diterpene ester, isolated from *Taxus brevifolia,* is the most promising antitumor agent developed around three decades ago and approved by the Food and Drug Administration (FDA) for the treatment of refractory ovarian cancer and breast cancer.

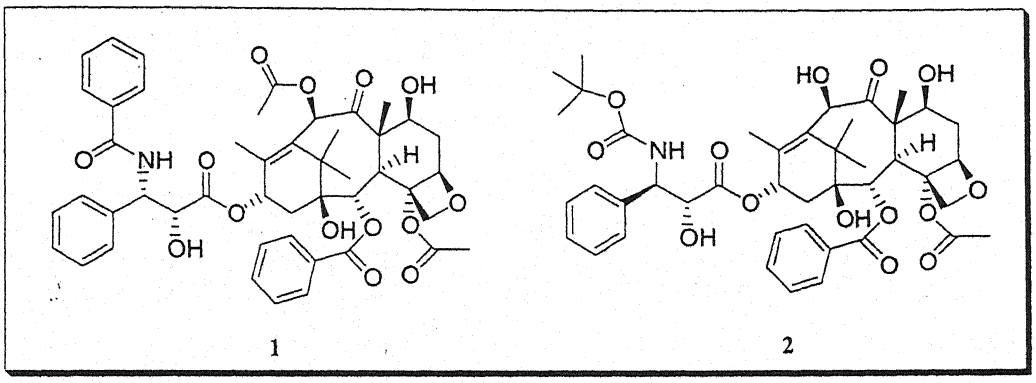


Figure 1: Most leading anti-cancer products

The most leading anticancer product, Paclitaxel (**1**) of Bristol-Meyers Squibb having recorded sales of $243 million in 2004, is active against a broad range of cancers that are generally considered to be refractory to conventional chemotherapy.[3,4] It binds strongly to tubulin and showed high toxicity against B-16 melanoma and is currently regarded as one of the best anticancer agent.[5] Later, during the development of the taxane derivatives, another potent analog appeared named as docetaxel (Taxotere) (**2**)[6,7,8]. Docetaxel is around 2.5 fold more active in causing inhibition of cell replication and acts on S-phase as compared to paclitaxel. Paclitaxel also binds to tubulin and induces its polymerization and promoting stable microtubule formation as shown in Figure 1.[9]

Revolutionary discoveries in the field of molecular biology resulted in a number of new biological targets such as tyrosine kinases, farnesyltransferases, protein kinases, histone deacetylases, glutathione S transferases and DNA/RNA polymerases, which have been widely used for the designing of new anticancer agents. However, tyrosine kinases have been successfully exploited in cancer chemotherapy and four drugs namely Irresa (**3**), Tarceva (**4**), Gleevec (**5**) and Canertinib (**6**), as its inhibitors, reached to the market. Moreover, several small synthetic molecules as tyrosine kinase inhibitor are in clinical development e.g., PTK787/ZK 222584 (**7**) and SU11248 (**8**) for the treatment of human cancers as shown in Figure 2.[10] Around twenty molecules, as tyrosine kinase inhibitors, are

currently in different clinical phases.[11] In addition, approximately twenty-eight molecules, as inhibitors of other targets described above, are at various stages of clinical trials. Despite these discoveries, new approaches required for major improvement of therapeutic agents. Therefore, new broad-spectrum cytotoxic analogs and target specific agents are being continuously synthesized worldwide in order to find anticancer agents with lesser toxicity and more potency as well as efficacy.

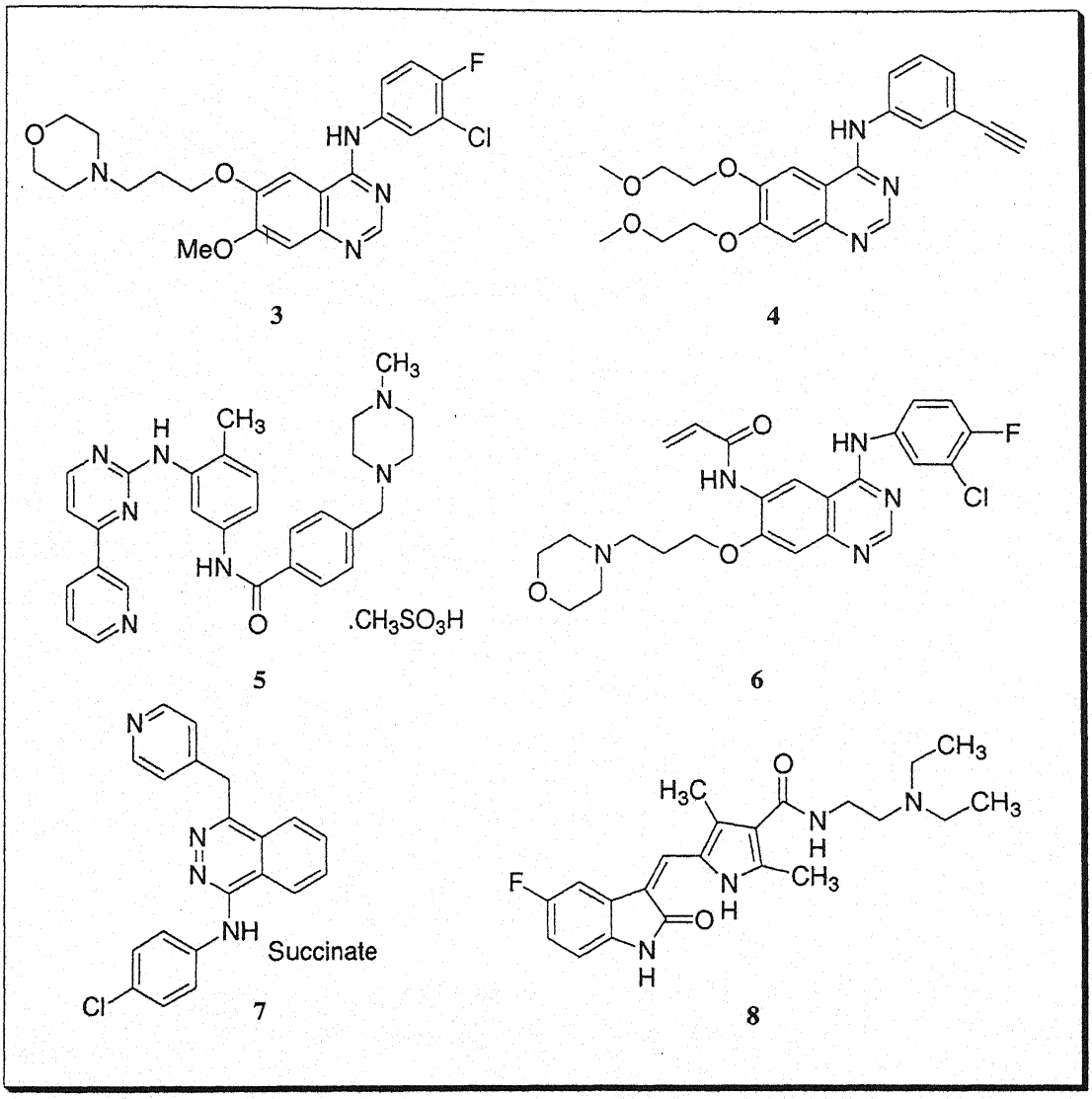


Figure 2: New anticancer agents

1.3. Betulinic acid as a Potential Anticancer Agent:

Natural products played a major role in the anticancer drug discovery. Over 60% of the anticancer drugs are of natural origin.[12] Betulinic acid (**9**) as shown in Figure 3, 3β-hydroxy-lup-20(29)-en-28-oic acid, a naturally occurring pentacyclic lupane type triterpene, is widely distributed throughout the tropics.[13] Betulinic acid is

isolated from various plants such as *Tryphyllum peltatum, Ancistrocladus heyneaus, Diospyrus spp.,*[14] *Tetracera boliviana, Ziziphus spp.*[15] and *Syzygium farmosanum*, etc. A variety of biological properties ascribed to betulinic acid such as anticancer, anti-inflammatory, antiviral, antiseptic, antimalarial, spermicidal, antimicrobial, antileshmanial, antihelmentic and antifeedent activities,[16,17] although the clinical activity has not yet been established.

However, betulinic acid was recognised for its anticancer and anti-HIV activities.[18, 19] Previous reports revealed that betulinic acid is a melanoma specific cytotoxic agent.[20] Recent evidence indicated that betulinic acid also possesses a broader spectrum of cytotoxic activity against other cancer cell lines. Betulinic acid has shown to function through the induction of apoptosis irrespective of the cells p-53 and CD-95 status.[21,22,23] Some experimental reports indicated that betulinic acid acts through the mitochondrial pathway,[24,25] though the precise molecular target and mechanism of action are not yet clear and is now the focus for number of ongoing research programs.[26] As far as toxicity of betulinic acid is concerned, it has been found highly safe even at the dose of 500 mg/Kg body weight. Therefore, these findings and favourable therapeutic index, made betulinic acid a very attractive agent for the clinical treatment for various types of cancers.

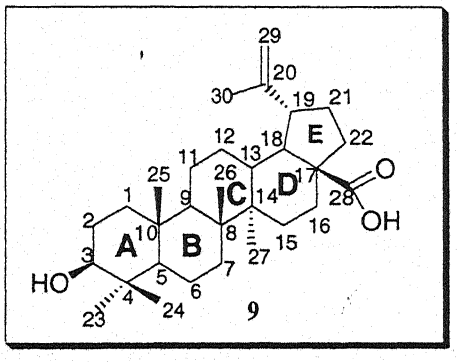


Figure 3: Betulinic acid

The *in-vitro* cytotoxicity activity (IC_{50} values) of betulinic acid has been summarized herein. The IC_{50} values of betulinic acid in melanoma cancer cell lines (SKMEL-2, SKMEL-3, UACC257, UISO-Mel-1, UISO-Mel-2 and UISO-Mel-4) are found in range of 0.69 to 4.8 μg/mL, while in breast cancer cell lines (BC1, BT549 and MCF7), IC_{50} values were found >20 μg/mL.[27,28] In colon cell lines (Caco2, COL2, HCT116, LNCaP and SW620), IC_{50} values of betulinic acid were found in the range of 1.23 to >20 μg/mL. In lung cancer cell lines (LNCaP, A549 and LU1), IC_{50}

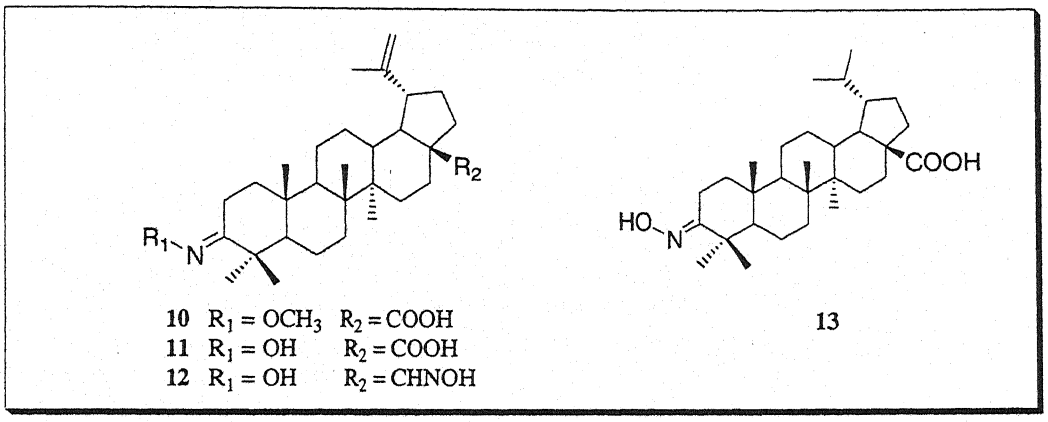
value were in the range of 7.7 to >20 μg/mL, while in giloblastoma cell lines (A172, SK14, SK17, SK19, SK22, SK37, SK51, SK55, SK60, U87MG, U118, U138, U251, U343 and U373), it was found in the range of 5 to >20 μg/mL. In medulloblstoma cancer cell lines (D283, D341, Daoy, MEB1, MHH1, MHH3 and MMH4), IC_{50} values were lying in between 3 to 15 μg/mL and in ovarian cancer cell lines (A2780, IGROV1 and OVCAR5), it varies from 1.8 to 4.5 μg/mL. In prostate cancer cell lines namely PC3 and KB, IC_{50} value was 1.9 and >20 μg/mL, respectively. It was, therefore, concluded that betulinic acid is a promising anticancer agent. And as a result, further studies have been performed to synthesize betulinic acid analogs in an effort to establish a meaningful structure activity relationship as well as to get more potent anticancer agents.

The C-1, C-2, C-3, C-4, C-20, C-28, A-ring, D-ring and E-ring are the positions for diversification in betulinic acid. Hundreds of derivatives prepared and tested for their cytotoxic activity. Herein, the structural modifications made at these positions in betulinic acid as well as their affect on the cytotoxicity, is compiled. We have assembled the active betulinic acid derivatives along with their cytotoxicity profile in each series.

1.3.1 Modifications at C-1, C-2 and C-3 in betulinic acid

The C-3 position in betulinic acid was widely exploited and hundreds of derivatives such as oxime, O-acyl, benzylidenes, hydrazine and hydrazones have been reported in the literature. A brief SAR in each of these series of compounds has been described herein. Kim *et al.* synthesized different C-3 oxime derivatives of betulinic acid (e.g., compounds **10-12**) as shown in Figure 4.[29] The loss of the activity was, however, observed in these compounds against MEL-2 cell line but exhibited interesting activity in KB cell line. Compound **12** was active against KB cell line (ED_{50} = 3.3 μg/mL), whereas slightly less active (ED_{50} = 2.2 μg/mL) on MEL-2 cell line than betulinic acid (ED_{50} = 1.2 μg/mL). In general, hydrogenation at C-20 position in C-3 modified derivatives does not show major difference in the anticancer activity in comparison to the corresponding non-hydrogenated molecules. Replacement of the C-28 carboxylic group by either oxime or alcohol or aldehyde or ester resulted loss in the activity. It seems that the free carboxylic at C-28 position is essential for eliciting cytotoxicity in C-3 oxime derivatives.

It is reported that betulinic acid inhibited one of the endogenous factor aminopeptidase-N and mitochondrial function in endothelial cells.[30] Based on these observations, different C-3 modified betulinic acid derivatives synthesized as antiangiogenic agents by Mukherjee *et al.* [31]



10 $R_1 = OCH_3$ $R_2 = COOH$
11 $R_1 = OH$ $R_2 = COOH$
12 $R_1 = OH$ $R_2 = CHNOH$

13

Figure 4: Derivatives of betulinic acid (compounds **10-13**)

Starting from 20, 29-dihydrobetulinic acid, hydroxyl oxime derivative **13** was obtained, which was found to be one of the most potent molecule against lung cancer cell lines such as L132 and A549 with ED_{50} of 1.5 and 1.8 μg/mL, respectively as shown in Figure 4. Compound **13** exhibited an ED_{50} of 1.1 μg/mL against DU145 cancer cell line and is comparable to betulinic acid (ED_{50} = 1.13 μg/mL). As discussed earlier for anticancer activity, in this case also, C-28 carboxylic acid appeared to play an important role in antiangiogenic activity in C-3 oxime derivatives. A number of 3-*O*- acyl derivatives (**14-17**) prepared by Mukherjee *et al.* as shown in Figure 5.[32] The 3-*O-acyl* betulinic acid derivative **14** showed IC_{50} 0.9 μg/mL on ECV304 (endothelial cell line) and low to moderate ECS (endothelial cell specificity). The 3-*O-acyl* dihydrobetulinic acid derivatives, **15** and **16**, exhibited better cytotoxicity (IC_{50} ~ 0.7 μg/mL) in comparison to betulinic acid (IC_{50} ~1.2 μg/mL) on ECV304 cell line. These compounds also showed slightly better ECS values than betulinic acid. Compound **16** showed better inhibition of TLS (tube like structure) formation than betulinic acid.

3-*O*-acetyl 20,29 dibromobetulinic acid (**17**) obtained upon the bromination of the acetylated betulinic acid, showed better activity (ED_{50} = 0.3 μg/mL) against U937 human histocytic lymphoma in comparison to betulinic acid (ED_{50} = 1.6 μg/mL). Thus, the bromo substituent at C-20 position in 3-*O*-acyl betulinic acid derivatives

played an important role in eliciting cytotoxicity.[31] Based on the structural similarity between betulinic acid and betulin, Kvasanica *et al.* recently prepared different 3-*O*-phthalic ester derivatives (**18-21**).[33] Several of them found to be more cytotoxic and polar in comparison to betulinic acid. Compounds **18** and **19** were prepared by transfer-hydrogenolysis of hemiphthalate of benzyl ester of betulinic acid. Both compounds **18** and **19** showed activity against a number of cancer cell lines like CEM, K562, K562-tax, HT29, PC-3 and SK MEL2.

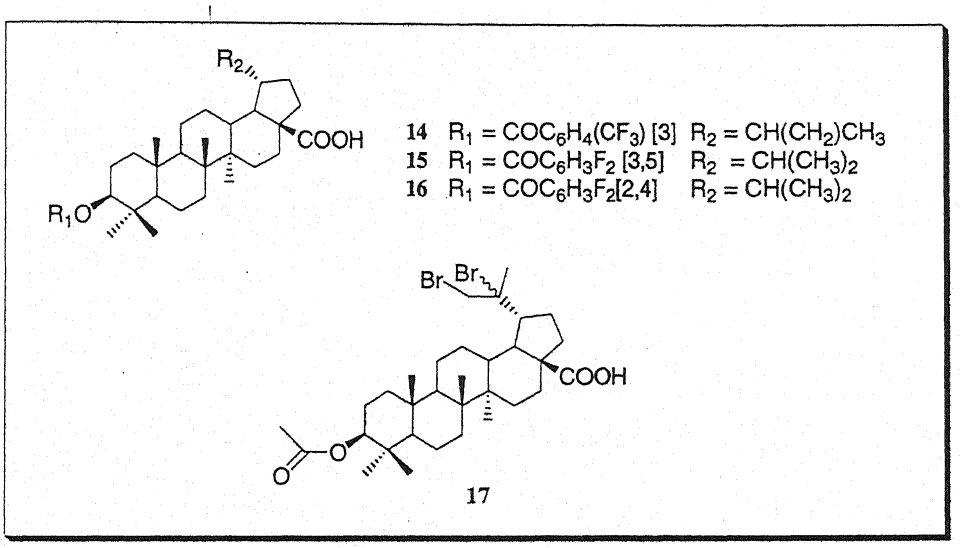


Figure 5: Derivatives of betulinic acid (compounds **14-17**)

Based on the structural similarity between betulinic acid and betulin, Kvasanica *et al.* recently prepared different 3-*O*-phthalic ester derivatives (**18-21**) as shown in Figure 6.[33] Several of them found to be more cytotoxic and polar in comparison to betulinic acid. Compounds **18** and **19** were prepared by transfer-hydrogenolysis of hemiphthalate of benzyl ester of betulinic acid. Both compounds **18** and **19** showed activity against a number of cancer cell lines like CEM, K562, K562-tax, HT29, PC-3 and SK MEL2. Compound **18** was found as a most active compound in this series with IC_{50} of 5.7, 8.8, 7.5 μg/mL on CEM, K562 and HT29 cancer cell lines, respectively, in comparison to betulinic acid (IC_{50} = 27.5, 54.8, 84.5 μg/mL in the same cell lines, respectively). Betulin that is inactive in nature, but its betulin phthalate ester derivatives **20** and **21** have shown good activity. Compound **20** obtained by mild basic hydrolysis of betulin diacetate, whereas **21** was prepared by selective acetylation of primary hydroxy group of betulin. Compounds **20** and **21** showed IC_{50} = 8.3 and 34.2 μg/mL, respectively, on K562-tax cancer cell line in comparison to betulinic acid (IC_{50} = 108.2 μg/mL). From SAR of these compounds, it

was concluded that derivatization of hydroxyl group at C-3 in betulinic acid by phthalic anhydride enhanced the cytotoxic activity. In particular, electron withdrawing group in the aromatic ring in 3-*O*-acyl group have enhanced the cytotoxicity with low to moderate ECS value, while bulky and electron donating group lowered the activity.

A number of benzylidene (e.g., **22**), hydrazine (e.g., **23** and **24**) and hydrazone (e.g., **25-28**) derivatives of betulinic acid have been reported as shown in Figure 6.[31,32] The 20, 29-dihydro-3 benzylidene betulinic acid derivative (**22**) was found highly cytotoxic (IC_{50} ~ 0.35 μg/mL) and showed good anti TLS property. Hydrazine derivatives, **23** and **24**, exhibited high cytotoxicity activity (IC_{50} ~ 0.5 and 0.4 μg/mL) on ECV304 cell line and high ECS value. It can be predicted that the introduction of electron donating group in aromatic ring at C-3 hydrazine functionality in 20, 29-dihydrobetulinic acid improved the activity. From SAR, it was concluded that protection of C-28 carboxylic acid in this series of betulinic acid or in dihydrobetulinic acid derivatives lowered the cytotoxicity.

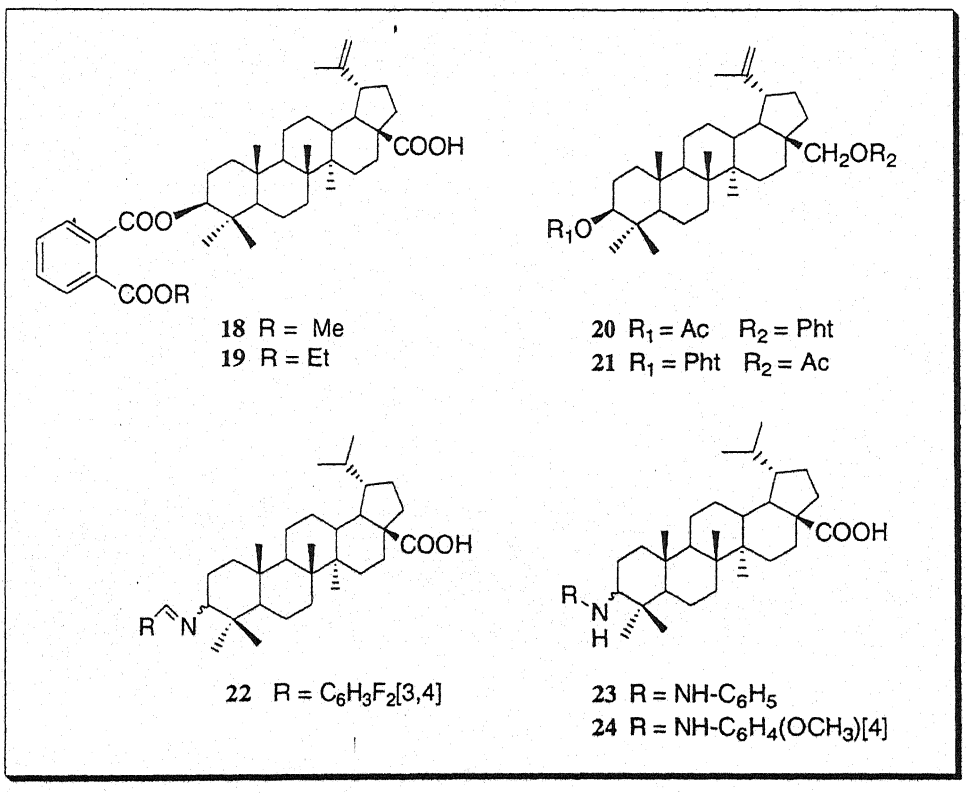


Figure 6: Derivatives of betulinic acid (compounds **18-24**)

The reaction of dihydrobetulinic acid with phenyl hydrazine furnished corresponding 3-phenyl hydrazone derivatives (e.g, **25-28**) as shown in Figure 7.

Compound **25** showed high order activity against DU145 and PA-1 cell lines with ED_{50} of 0.6 and 0.4 μg/mL, respectively, in comparison to betulinic acid (ED_{50} of 2.6 and 4.1 μg/mL).[31] Compounds **26-28** showed $IC_{50} < 0.4$ μg/mL on ECV cell line with moderate to high ECS value (ECS >10) against A-549 cell line. These betulinic acid derivatives inhibited around 13.1-49.2% of tube like structure formation (TLS) of ECV304 cells in a matrigel tube formation assay in comparison to 5.5% of betulinic acid.[26] It seems that hydrazone group at position-3 together with 20,29 dihydro moiety played a key role in endothelial cytotoxicity preferably in anti-TLS activity.

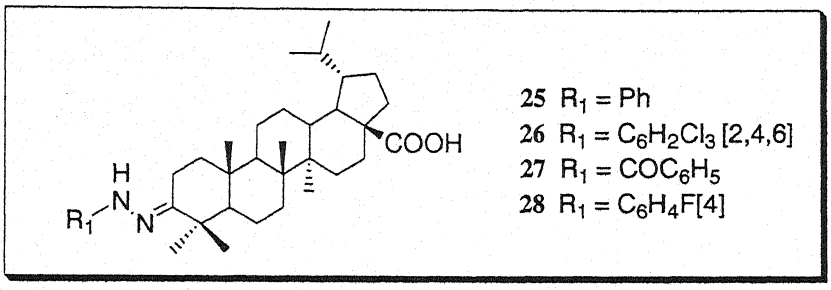


Figure 7: Derivatives of betulinic acid (compounds **25-28**)

You *et al.* synthesized several C-1 and C-2 modified betulinic acid derivatives (e.g., **29-31**) in which A ring is substituted by different electron-withdrawing group with least steric nature as shown in Figure 8.[34] For example, 3-*O*-1-ene moiety (in A ring) was introduced along with different groups at C-2 position, such as 2-cyano (compound **29**), 2-chloro (compound **30**) and 2-formyl (compound **31**).

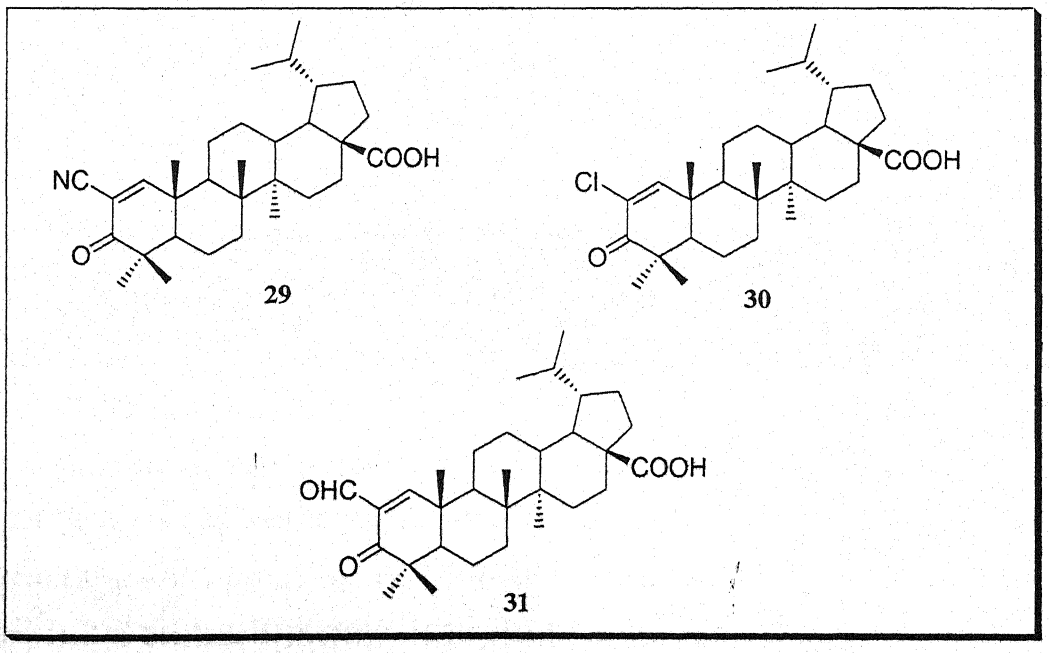


Figure 8: Derivatives of betulinic acid (compounds **29-31**)

The synthesis of compounds **29-31**, were performed in the following ways: betulonic ester was converted to 20-hydromethylene-3-oxo derivative followed by isoxazole ring formation and which upon ring opening and oxidation of the product thus obtained, furnished compound **29**. The 2-chloro (compound **30**) was obtained by converting C-28 protected dihydrobetulonic acid to an epoxy derivative followed by the acid-base treatment. Compound **31** was synthesized by converting 3-oxo-betulinic acid to hydroxymethylene derivative followed by subsequent oxidation and base treatments. These compounds were tested on different cell lines such as SK-MEL-2, A-549 and B16-F10 for their anticancer activity. Compound **30** was found most active in the series, as it was around 59 fold more potent than betulinic acid (ED_{50} = 0.13 μg/mL) on SK-MEL-2 cell line. Compound **29** exhibited higher cytotoxicity (ED_{50} = 0.81 μg/mL) in comparison to betulinic acid (ED_{50} = 7.62 μg/mL) on SK-MEL-2 cell line. Compound **31** (ED_{50} = 0.26 μg/mL) elicited 28 times higher efficacy than betulinic acid. It was interesting to note that in this case, double bond at C-20 position was not essential for cytotoxicity activity. The C-28 carboxylic acid was, however, critical for the cytotoxic activity, but in few cases methyl ester also found active. Thus, it is predicted that the derivatives with C-28 carboxylic acid and electron withdrawing at C-2 would show strong cytotoxicity.

Urban *et al.* synthesized several diosphenols and seco derivatives of betulinic acid (e.g., **32** and **33**).[35] For the synthesis of diosphenol derivative (**32**), betulinic acid was oxidized to 3-*O*-betulinic acid, which upon further oxidation provided compound **32**. Dimethylation of compound **32** furnished **33**. Cytotoxicity of these compounds were checked in several cell lines such as CEM, HT 29, K562, K562 Tax, PC-3, A549, DU 145 MCF 7 and SK-Mel2. Both the compounds **32** and **33** were highly cytotoxic on CEM cell line with IC_{50} of 4 and 5 μmol/L, respectively, in comparison to betulinic acid (IC_{50} = 27 μmol/L) as shown in Figure 9.

Very few anticancer compounds are known to be active against the tumour cell, endothelial cells and simultaneously act as angiogenesis inhibitors. Mukherjee *et al.* synthesized several 2-bromo derivatives, which exhibited potent activity on endothelial cells as well as tumor cells.[26] For example, compound **34** was highly potent (IC_{50} ~ 0.27 μg/mL) on ECV 304 cell line and having moderate ECS value as compared to betulinic acid (IC_{50} ~ 1.26 μg/mL) as shown in Figure 9. In general, the halo substituent in C-2 modified derivatives have improved the cytotoxicity.

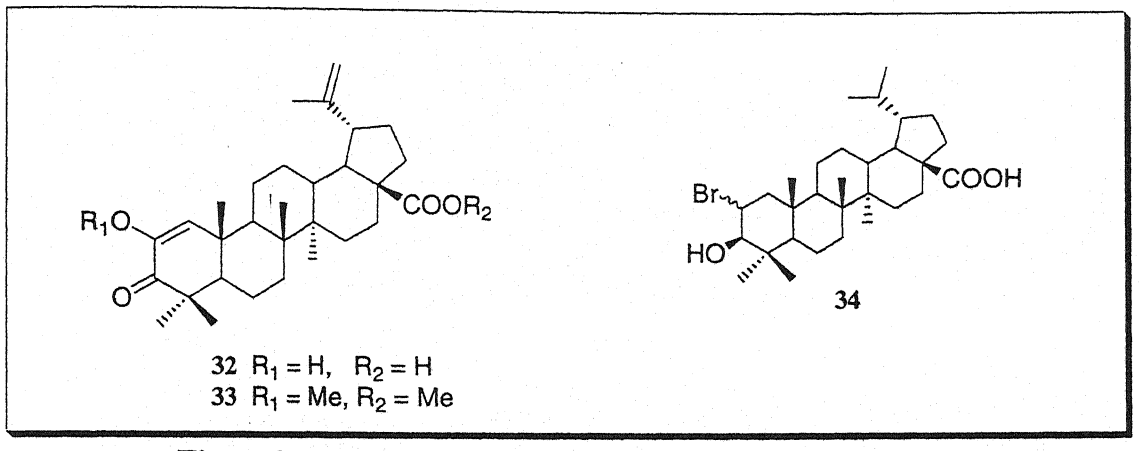


Figure 9: Derivatives of betulinic acid (compounds **32-34**)

1.3.2 Modifications at C-1, C-2, C-3 and C-4 in betulinic acid

Several C-1, C-2, C-3 and C-4 modified betulinic acid derivatives synthesized and screened for cytotoxicity and the SAR results are summarized here. Remangilones (**35-38**) are the cytotoxic compounds obtained from the plant *Physena medagascariensisl* as shown in Figure 10.

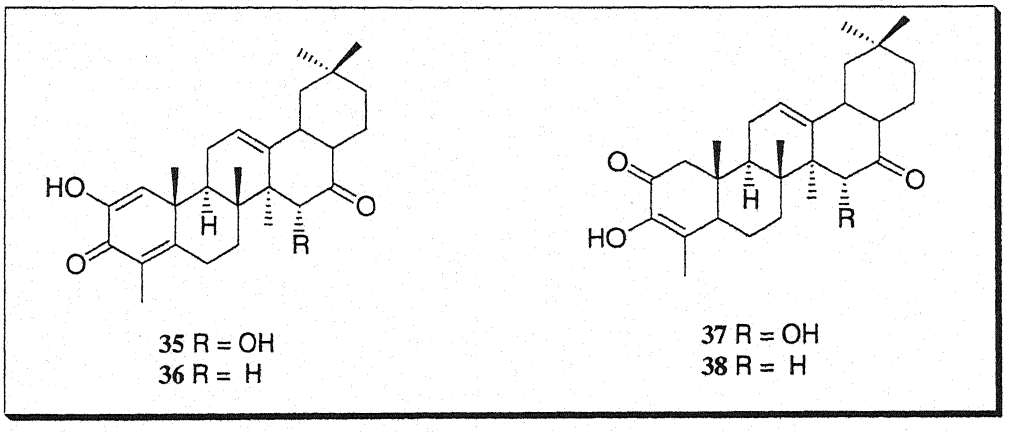


Figure 10: Derivatives of remangilones (compounds **35-38**)

Deng *et al.* converted betulin (compound **39**) into the 24-nor analogous (compound **40**), possess the same ring system as in remangilones as shown in Figure 11.[36] Compound **40** was synthesized in the following manner. Ring A of betulin was opened under harshed condition using Suarez cleavage and an extra carbon at C-24 position was then removed by oxidative cleavage. The A ring reclosure was carried out by SmI_2 mediated pinacol coupling to afford compound **40**. Compound **41** was prepared by the oxidation of hydrogenated betulin and followed by its conversion to methyl ester and then silylation and demethylation. Both these compounds (**40** and

41) were screened for their biological activity and calculated GI_{50}, TGI and LC_{50} values. These compounds were found slightly less active in comparison to betulinic acid. Compound **40** showed GI_{50} value < 10 nM on a particular ovarian cancer cell line (SK-OV-3). Whereas, **41** exhibited GI_{50} activity in range of 2-7 μM activity on six leukemia cell lines.

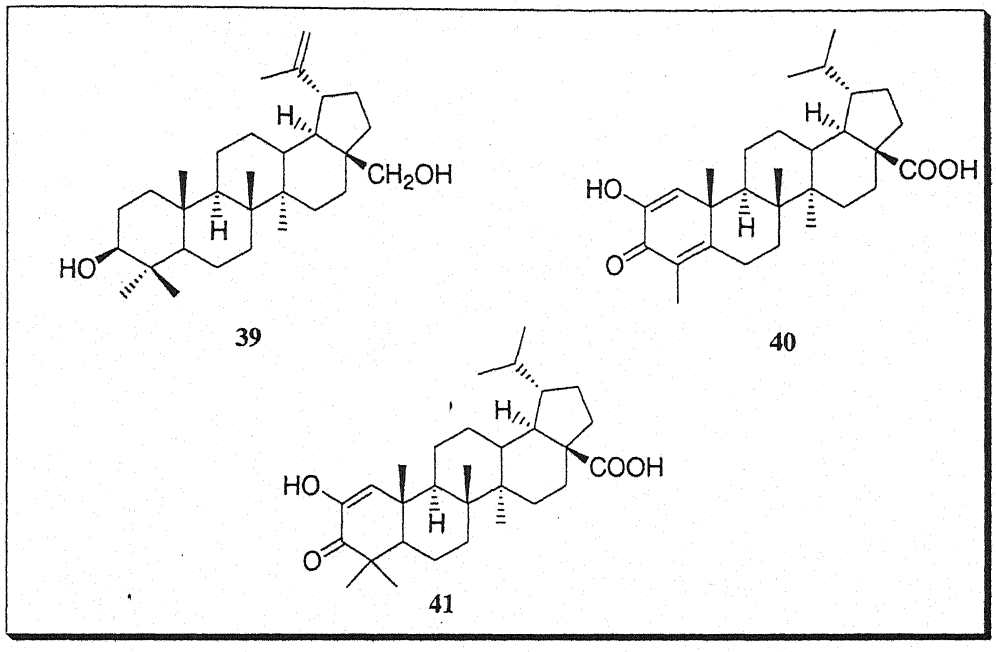


Figure 11: Derivatives of betulinic acid (compounds **39-41**)

1.3.3 Modifications at C-20 in betulinic acid

Kim *et al.* carried out several modifications at alkene group at C-20 position in betulinic acid (e.g., compounds **42-45**) as shown in Figure 12.[37]

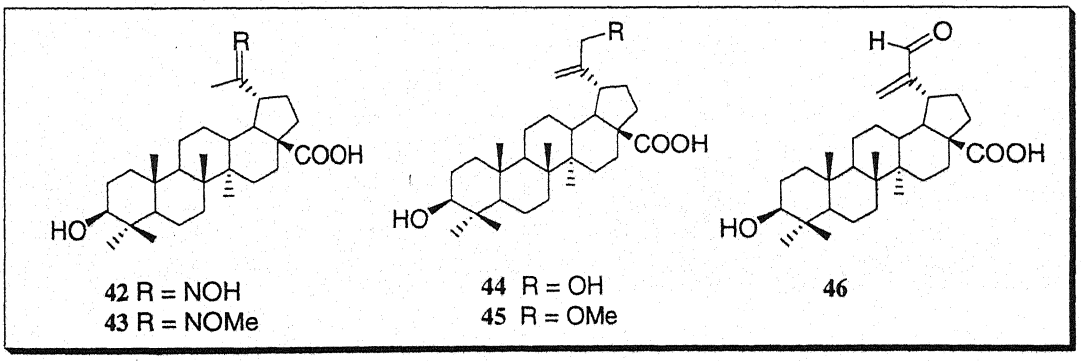


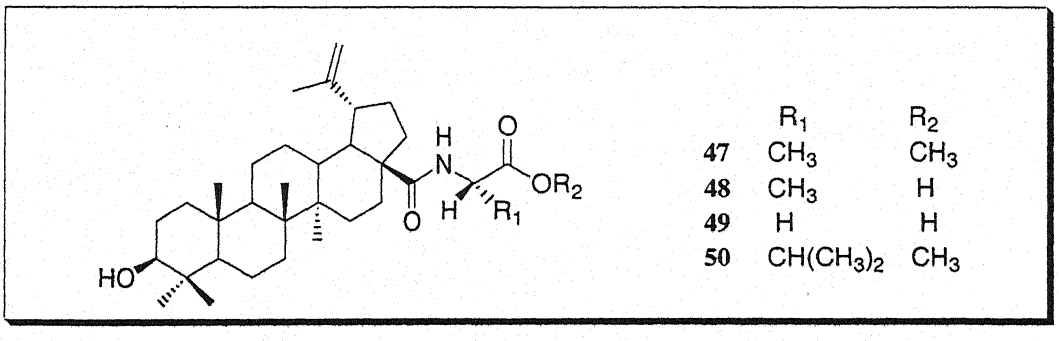
Figure 12: Derivatives of betulinic acid (compounds **42-46**)

The C-20 position was found to be sensitive to size and electron density of the substituents in retaining the cytotoxicity. The chemical modification at C-20 was carried out by converting it into ketone followed by the treatment with different

hydroxylamines to get corresponding oximino derivatives (compounds **42** and **43**). Oximes appeared to result in loss of cytotoxicity. Also upon reduction of keto group to secondary alcohol resulted to an inactive compound. Other derivatives like compounds **44** (primary alcohol) and **45** (methoxy ether) were synthesized by converting C-29 position of 3-acetybetulinic acid to bromo derivative and which, upon further reaction with desired substituent followed by Compound **46** showed TCS_{50} (concentration with 50% tumor cell survival) 4.0 μM on CEM cancer cell line. This compound also showed accumulation of cells in G2/M and S region of cell cycle. The above result showed the role of size and electrostatic effect at C-20 position in betulinic acid. Therefore, C-20 was found to be undesirable position in betulinic acid for derivatization. However, the cytotoxic activity of the compound **46** is assigned due to the presence of α-unsaturated keto group.

1.3.4 Modifications at C-28 in betulinic acid

As discussed earlier, betulinic acid displayed a good anticancer activity but problem associated with its water solubility. Jeong *et al.* produced the C-28 amino acid conjugates (**47-50**), which caused improvement in selective toxicity as well as water solubility of betulinic acid as shown Figure 13.[39]



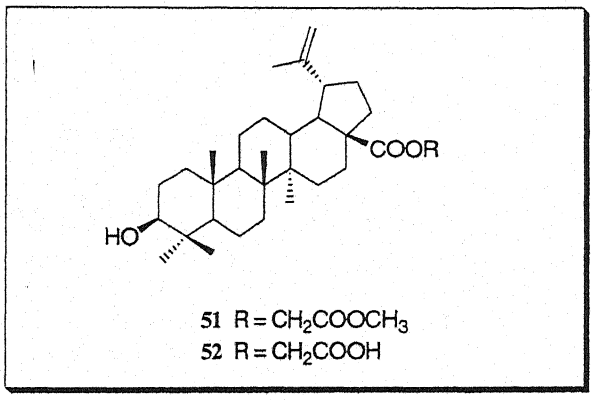
	R_1	R_2
47	CH_3	CH_3
48	CH_3	H
49	H	H
50	$CH(CH_3)_2$	CH_3

Figure 13: Derivatives of betulinic acid (compounds **47-50**)

Activity of the compounds checked in human melanoma (MEL-2) and fibrosarcoma (KB) cell lines. In case of alanine methyl ester (compound **47**), ED_{50} was 3.5 μg/mL on MEL-2 cell line while its free acid, compound **48**, was found effective against both MEL-2 and KB cell lines with ED_{50} of 1.5 and 4.6 μg/mL, respectively. Compound **48** was found to be better in comparison to betulinic acid (ED_{50} = 4.2 and >20 μg/mL in the same cell line, respectively) and simultaneously,

improved the water solubility. The free acid of glycine (compound **49**) was effective on MEL-2 cell line similar to betulinic acid with ED_{50} of 4.2 μg/mL but showed the best water solubility (2 mg of **48** was dissolved in 200 μL of DMSO and upon dilution of 20 μL of this solution with distilled water provided water solubility of 100x). ED_{50} of methyl ester of valine (compound **50**) was found to be 2.1 μg/mL. It is observed that glycine conjugate of betulinic acid had improved the solubility without affecting the cytotoxicity.

Ramadoss *et al.* synthesized various C-28 ester derivatives of betulinic acid, and in general, cytotoxicity did not improve.[40,41] However, compounds **51** and **52** showed selective cytotoxicity on PA-1 (human ovary) cancer cell line with ED_{50} of 3.1 and 1.3 μg/mL, respectively, better than betulinic acid (ED_{50} >10 μg/mL).



51 R = CH_2COOCH_3
52 R = CH_2COOH

Figure 14: Derivatives of betulinic acid (compounds **51-52**)

1.3.5 Ring A expansion in betulinic acid

Isoxazole fused (**53**) and seven membered (**54** and **55**) derivatives were prepared and screened for the cytotoxic activity. You *et al.* synthesized compound **53** by converting 3-oxo betulinic acid into hydroxymethylene derivative, which upon condensation provided isoxazole ring as shown in Figure 15.[34]

This compound showed good cytotoxicity against A-549 cell line with ED_{50} of 1.54 μg/mL, which is around 5 times more than betulinic acid.

Urban *et al.* synthesized seco derivatives (**54** and **55**) of betulinic acid as shown in Figure 15.[35] After converting betulinic acid to betulonic acid, the latter was oxidized to give diosphenol compound, which was then cleaved to seco derivatives and changed to seven membered cyclic anhydrides (**54** and **55**).

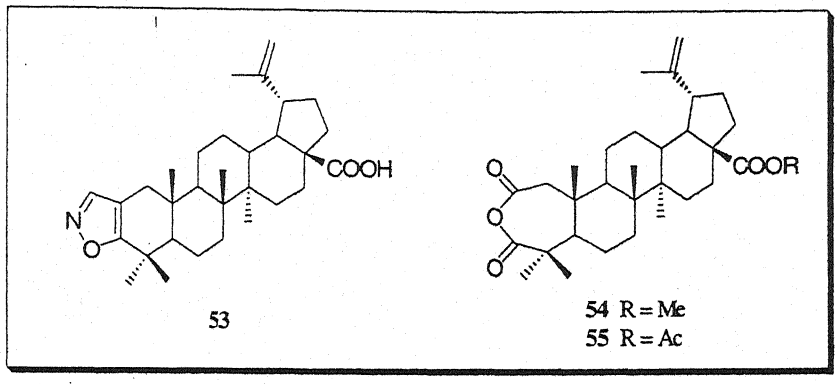


Figure 15: Derivatives of betulinic acid (compounds **53-55**)

Compounds **54** and **55** showed the maximum activity on CEM cancer cell line with IC_{50} of 7 μmol/L and 6 μmol/L, respectively, in comparison to betulinic acid (IC_{50} 27 μmol/L).

1.3.6 Ring E modifications in betulinic acid

Sarek *et al.* modified E ring in betulinic acid with different oxygen functions (compounds **57** and **58**), which were given a common name betulinines.[38] Starting compound **56** (21-oxolup-18-ene-3β, 28-diyl diacetate) was obtained by various chemical modifications of betulin. Compound **56** upon selective saponificatin and oxidation, gave rise to diketone compound **57**. Compound **56** upon oxidation and breaking of the 18,19 double bond gave rise to a tetraketone, which upon further cleavage afforded carboxylic acid derivative **58** as shown in Figure 16.

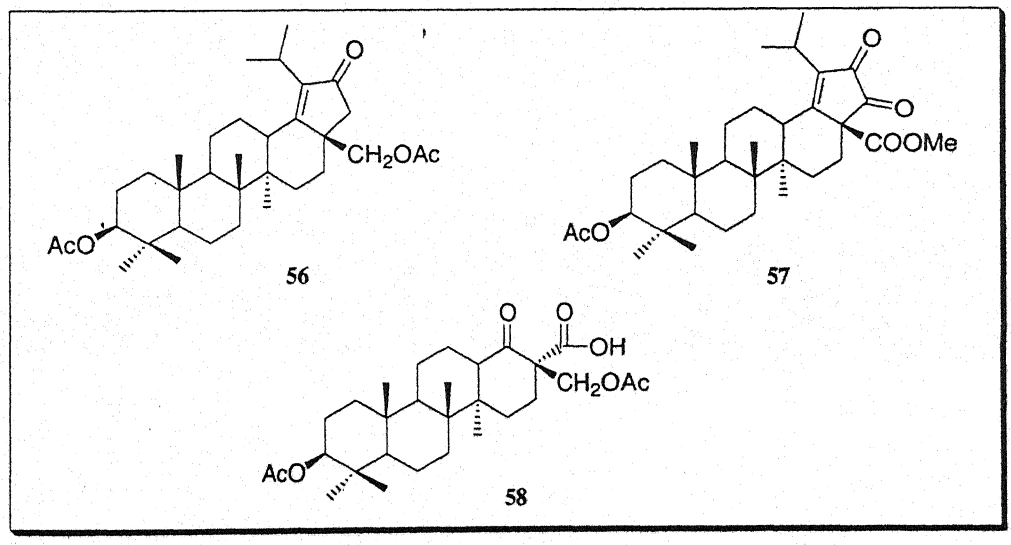


Figure 16: Derivatives of betulinic acid (compounds **56-58**)

Anticancer activities of these compounds were tested on CEM, epithelial, neuroectodermal and mesodermal tumor cell lines. Apoptosis in CEM cell line was shown by flow cytometry and scanning electron microscopy techniques. Both the compounds **57** and **58** showed, TCS_{50} of 4.4 and 1.0 μM, respectively on CEM cell line. Compound **58** was found as the most active compound in this series. These derivatives possess broad-spectrum anticancer potency. This compound (**58**) was effective in both drug resistant and drug independent cell lines. Therefore, these molecules may show a good scope for cancer chemotherapy resistant persons. Acetoxymethyl group at C-17 position in compound **58** provided stability due to its steric nature and prevents formation of six membered ring.

1.3.7 D and E rings modifications in betulinic acid

Favaloro *et al.* synthesized tricyclic compound **59**, which showed significant inhibitory activity (IC_{50} ~ 0.01 μM) on nitric oxide synthase inhibition, is related to anticancer activity as shown in figure 17.[42] Further modification of this compound (**59**) was carried out by induction of electron withdrawing group at C-13 position gave rise to a more potent compound **60** (IC_{50} ~ 2.1 nM). This indicated that these tricyclic molecules might provide a new molecular skeleton to design broad-spectrum anticancer agents. The low molecular weight compounds are always preferred in clinics.

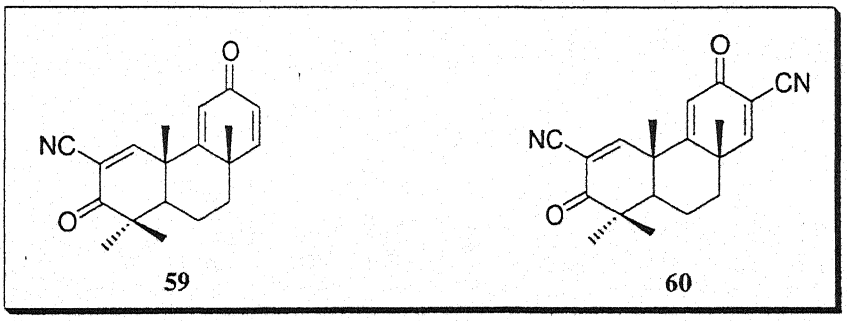


Figure 17: Tricyclic derivatives (compound **59-60**)

As targeted cancer therapy has been rapidly expanding and small organic molecules are being favored in clinics. Amongst small synthetic organic molecules, quinazoline was identified as one of the most successful chemical class. Based on the success of quinazolines in cancer chemotherapy, more recently, medicinal chemists have attracted towards its closely related chemical classes such as quinolines and naphthyridines. These chemical classes are now being explored in cancer chemotherapy and several molecules have already reached in advanced clinical trials.

1.4 Quinazoline Derivatives as Anticancer

The receptor tyrosine kinase promotes the signal transduction in tumor cells or endothelial cells via the binding of the growth factors, which are EGF (epidermal growth factor), PDGF (platelet-derived growth factor) and VEGF (vascular endothelial growth factor). Various quinazoline-based derivatives have shown activity against tyrosine kinase and acts as potent EGFR, VEGFR and PDGFR inhibitors. Their structure activity relationship has been discussed here.

1.4.1 EGFR Inhibitors:

Bridges *et al.* synthesized various quinazoline derivatives of prototype **I** as EGFR inhibitors as shown in Figure **18**.[43] SAR studies showed that compounds with dimethoxy substituents at C-6 and C-7 are found to be active. The 4-α-phenylethylaminoquinazoline with (*R*)-methyl substituent showed potent activity, while its (*S*)-enantiomer exhibited considerably weaker inhibitory activity. Increasing the side chain longer than methyl at benzylic CH is strongly disfavored. Simultaneously, increasing the size of the aromatic ring at C-4 from phenyl to naphthyl also decreased the cytotoxicity.

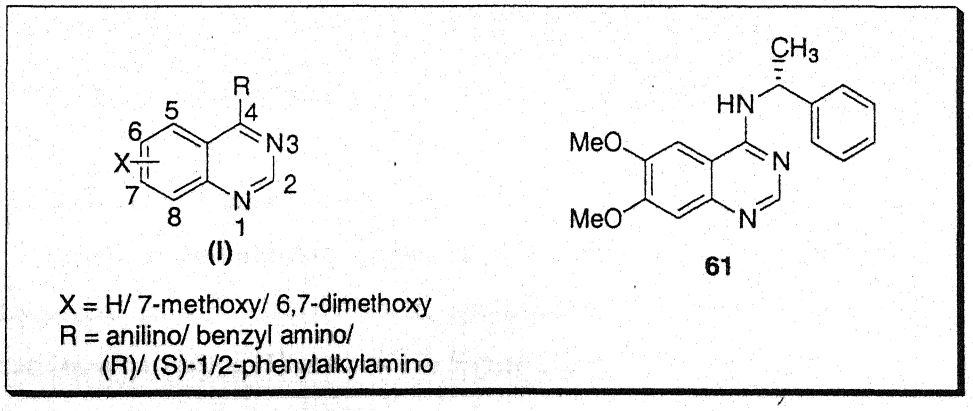
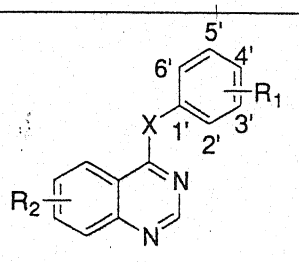


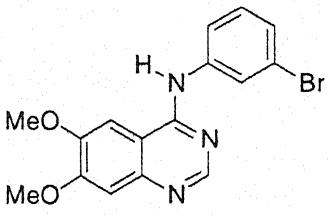
Figure 18: Quinazoline prototype **I** and derivative (Compound **61**)

However, compound **61** was found the most active with IC_{50} of 1.6 nM for EGFR tyrosine kinase. Lineweaver-Burke analysis showed that compound **61** acts as a clean reversible ATP-competitive inhibitor with *Ki* of 627 pM. It was predicted that (*R*)-methyl group of compound **61** binds precisely in a small depression on the enzyme, while the (*S*)- methyl group causing unfavorable steric interactions with the other surface of the cleft.

Rewcastle *et al.* synthesized a number of 4-substituted quinazolines of prototype **II** as inhibitor of tyrosine kinase activity of epidermal growth factor receptor as shown in Figure **19**.[44]



(II)



62

X = O/ NH/ aminoalkyl/ R_1 = H/ halo/ methoxy/ N_2/
R_2 = 5-NO_2/ 5-NH_2/ 5-OMe/ 6-NO_2/ 6-NH_2/ 6-OMe/ 7-NO_2/ 7-NH_2/ 7-OMe/ 6,7-di-OH/ 6,7-di-NH_2/ 6,7-di-OMe

Figure 19: Quinazoline prototype **II** and derivative (Compound **62**)

SAR showed that the nature of the linking group between the quinazoline ring and phenyl side chain has the substantial effect on the inhibitory activity. Small lipophilic and electron withdrawing group at C-3 in phenyl ring has showed the potent activity. It was interesting to note that alteration in the positions of nitrogen pattern in quinazoline ring leads to inactive compounds. Substitution of the quinazoline ring with electron donating group improved the potency. Compound **62** is identified as a highly selective inhibitor of the tyrosine kinase and showed competitive inhibition with respect to ATP. Compound **62** has shown IC_{50} of 0.029 nM against the isolated enzyme of EGFR and IC_{50} of 15 nM for inhibition of EGF-stimulated tyrosine phosphorylation in NIH3T3 cells.

Based on the inhibitory properties of 4-anilinoquinazoline **62** against EGFR, as discussed above,[45] Bridges *et al.* synthesized a number of new quinazoline derivatives of prototype **III** as shown in Figure 20.

SAR studies showed that, the compounds having halogen substituent at C-3 in aniline ring exhibited activity. Replacement of halogen group in aniline ring showed steric tolerance of the aniline-binding site. The supraadditive effect was observed in 4-(3'-bromoanilino)quinazoline derivatives. It was also observed that those compounds, which are similar to compound **62**, did not show activity. So, only appropriately substituted quinazolines possess the ability to induce a change in the confirmation of the tyrosine kinase domain. The 6,7-ethoxy derivative **63** showed IC_{50} of 0.006 nM, while dimethoxy congener **62** with had shown IC_{50} of 0.025 nM against EGFR.

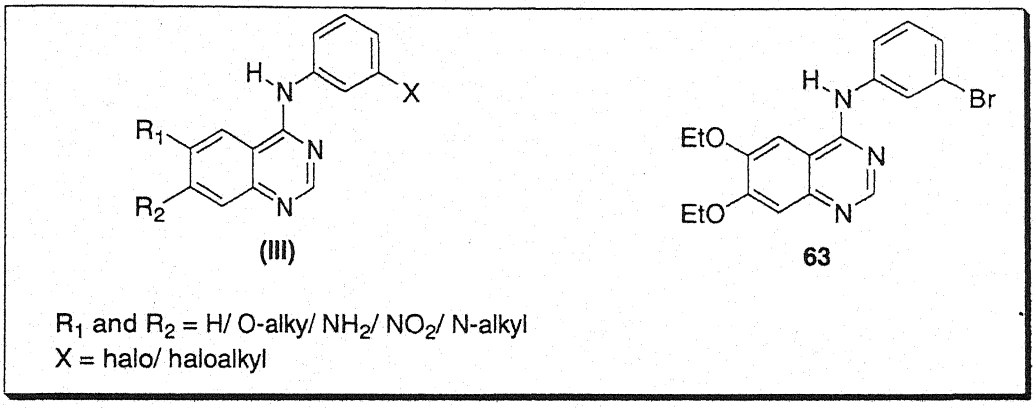


Figure 20: Quinazoline prototype **III** and derivative (Compound **63**)

It was observed that, electron-donating substituents (like dimethoxy or hydroxy or amino groups) at C-6 and C-7 in quinazoline ring give rise to active compounds but the dimethoxy substituted quinazolines have shown relatively better activity profile. Based on these observations, Rewcastle *et al.* generated additional a 5- and 6-membered ring across C-6/ C-7 and C-5/C-6 positions. The fused tricyclic analogues of prototypes **IV**, **V** and **VI** were synthesized without increasing the bulk at C-5, C-6 and C-7 positions as shown in Figure 21.[46]

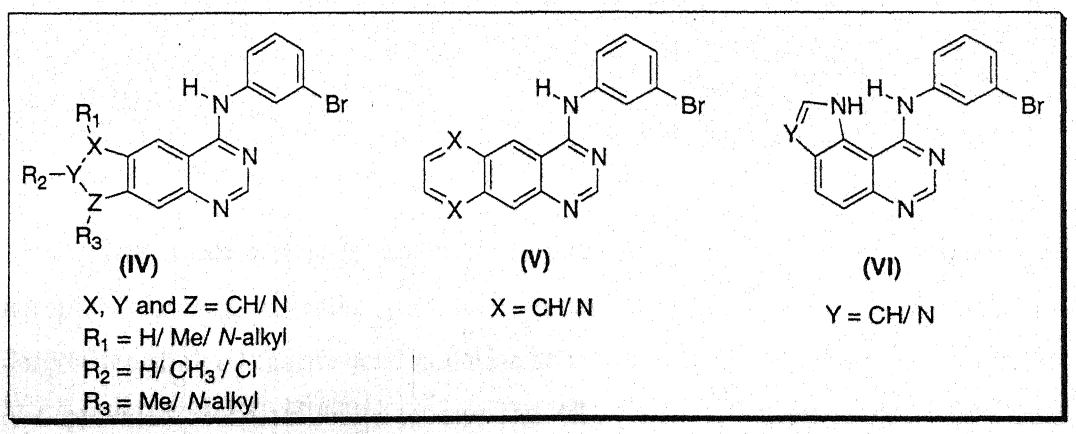


Figure 21: Quinazoline prototypes **IV**, **V** and **VI**

SAR showed angular imidazoloquinazolines is less effective inhibitor than the linear isomers. Linear imidazolo-, pyrazolo- and pyrroloquinazoline compounds (**64-67**) have exhibited potent activity as shown in Figure 22. Amongst them, linear imidazoloquinazoline **64** was found as most potent EGFR inhibitor. Compound **64** showed IC_{50} of 0.008 nM against phosphorylation caused by EGFR. Compound **64** is a potent inhibitor (IC_{50} of 46 nM) of autotophosphorylation of the EGFR in EGF-stimulated A431 cells. Compound **64** also exhibited similar activity against Swiss 3T3 cells. While compounds **65-67** showed IC_{50} of 0.34, 0.44 and 0.44 nM, respectively for EGFR inhibition.

Palmer *et al.* carried out the synthesis of various tricyclic quinazoline derivatives of prototype **VII**.[47] This study was based on the linear tricyclic quinazoline molecules **66** and **67** reported by Rewcastle *et al.*, which had shown poor aqueous solubility.[46] Based on the molecular study and structure activity relationship, more soluble tricyclic analogues were synthesized.

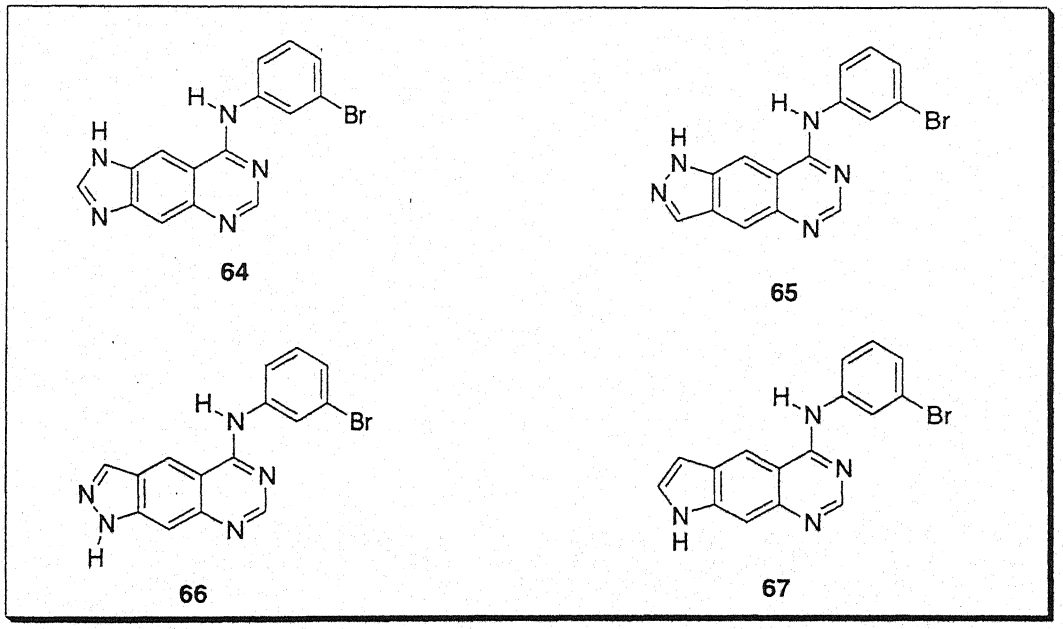


Figure 22: Derivatives of Quinazoline (Compound **64-67**)

SAR studies showed that both quinazoline nitrogens act as hydrogen bond acceptor and are essential for the activity. *N*-substituted pyrazoloquinazoline derivatives showed that H-bond donation is not critical. Stronger basic side chain at *N*-1 provided more solubility (compound **68**), while the weaker basic side chain analogues are more active (compound **69**) as shown in Figure 23.

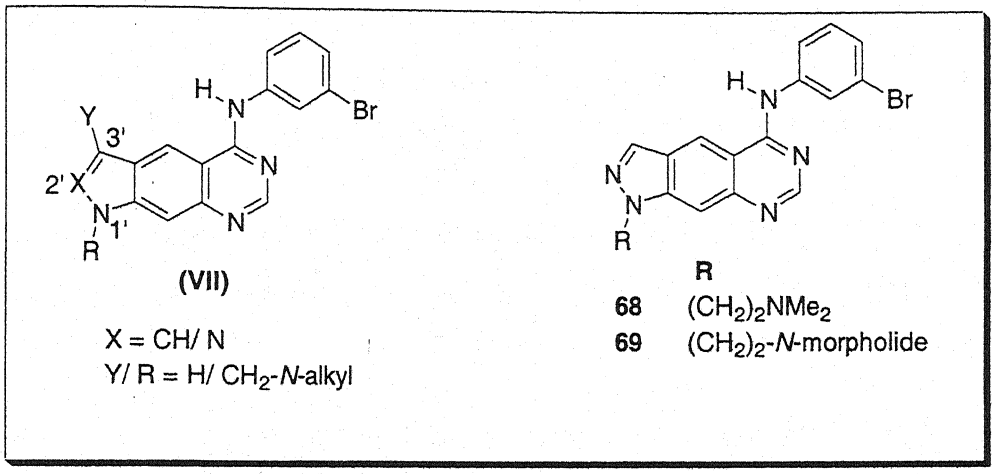


Figure 23: Quinazoline prototype **VII** and derivatives (Compound **68-69**)

Pyrroloquinazoline derivatives were, in general, more soluble than pyrazolo ones. Strongly basic side chains in pyrroloquinazolines lead to loss of potency while the weakly basic chain (compound **70**) showed good activity. The C-3' substituted pyrroloquinazoline derivatives (compounds **71-74**) were most effective with an average IC_{50} of 4 nM on EGFR as shown in Figure 24.

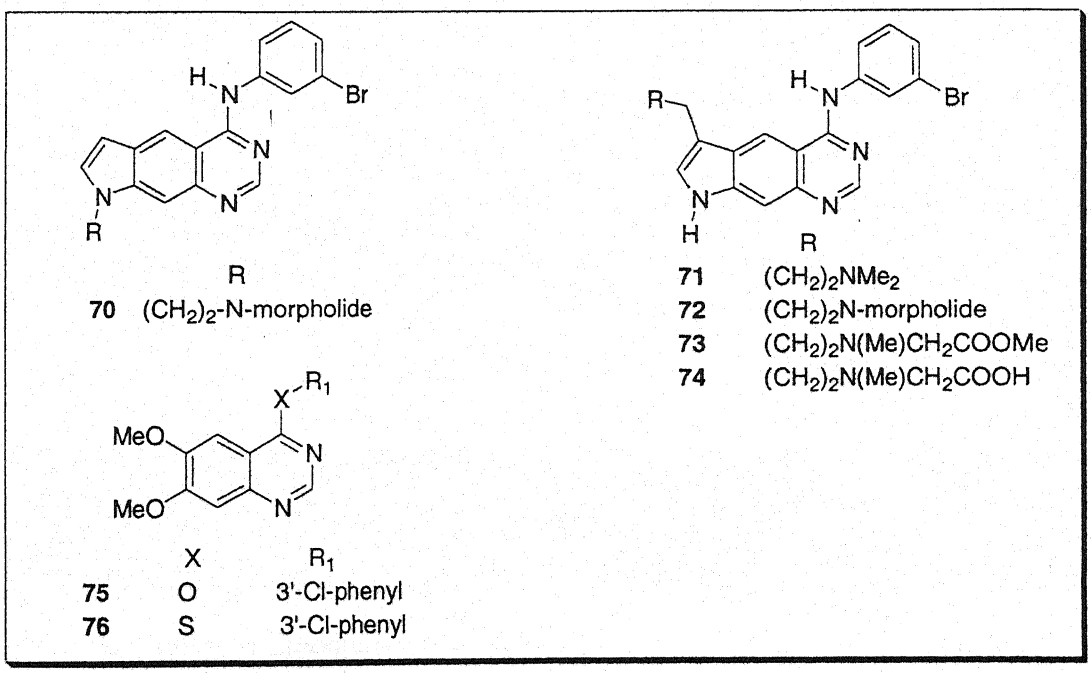
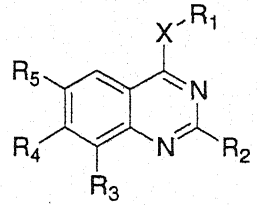


Figure 24: Derivatives of quinazoline (Compound **68-69**)

Compound **74** was found to be the most potent derivative with IC_{50} ~ 0.72 nM. These compounds were also evaluated for their ability to inhibit the autophosphorylation of EGFR in EGF stimulated A431 cells in culture. The pyrroloquinazoline derivative **70**

was the most potent against the isolated enzyme with IC_{50} ~ 16 nM, while the parent compounds **71** and **72** showed IC_{50} ~ 20 nM. In general, it seemed that pyrroloquinazolines have shown better profile than pyrazoloquinazoline.

Myers *et al.* synthesized C-2/C-4/C-6/C-7/C-8 substituted quinazolines of prototype **VIII** as EGFR and p56lck inhibitors as shown in Figure 25.[48] The p56lck inhibitors are used in the treatment of autoimmune diseases, while EGFR inhibitors are related to anticancer activity.[49]



(VIII)

R_1 = aryl/ 3-haloaryl/ 4-hydroxyaryl/ 3,4-dimethoxyaryl/ 3,4,5-trimethoxyaryl
R_2 = H/ halo
R_3 = H/ alkyl/ alkoxy
R_4 = H/ alkoxy
R_5 = H/ halo/ alkoxy
X = O/ S/ NH

Figure 25: Quinazoline prototype **VIII**

SAR for EGFR inhibitors showed that C-3'chloro substituent in phenyl ring at R_1 was found relatively better amongst other halo substituents. Upon replacement of nitrogen linker in 3'-chlorophenyl derived quinazolines by oxygen or sulfur causes small improvement in the activity but it reduced the activity in the compounds, which do not possess 3'-chlorophenyl group. The presence of electron donating group in phenyl ring at R_1 did not increase the activity. Quinazoline derivative possessing halo or alkyl substituents at C-2 and C-8 positions showed reduced activity, which indicates, and which showed the importance of steric hindrance in the N-1 interaction with the enzyme. However, compounds **75** and **76** were identified as most active EGFR inhibitors. Compounds **75** and **76** showed IC_{50} of 0.02 and 0.03 μM against EGFR autophosphorylation respectively.

Till 1997, anilinoquinazoline had been well-recognized chemical class for having activity against the EGF-RTK enzyme and compound **77** has been identified as a potent inhibitor of EGF-RTK. It showed in vitro activity of IC_{50} ~ 0.009 μM against A431 cancer cell line and 0.08 μM on KB cancer cell line. Based on these findings, Gibson *et al.* had introduced different linking groups in between quinazoline and phenyl rings at C-4 position (prototype **X**) and also generated additional rings in

between nitrogen linker and phenyl ring (prototype **IX**, **XI** and **XII**) as shown in Figure 26.[50]

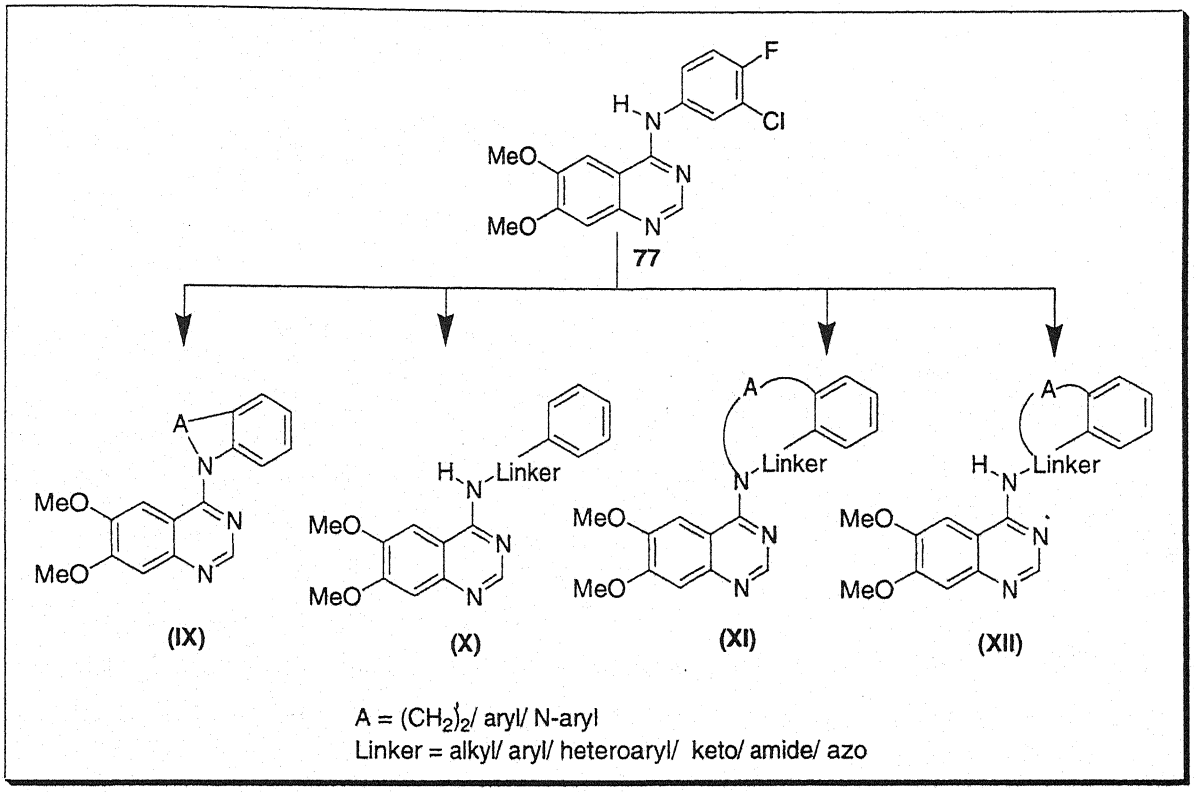


Figure 26: Quinazoline Prototypes **IX**, **X**, **XI** and **XII**

SAR studies showed that modification in prototypes **X** and **XII** provided the most active derivatives such as compounds **78** and **79** as shown in Figure 27.

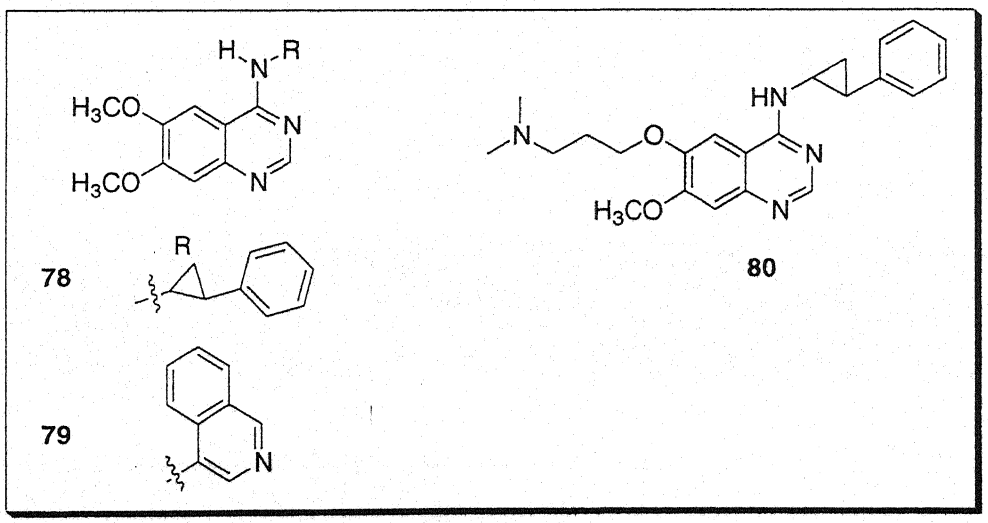


Figure 27: Derivatives of quinazoline (Compound **78-80**)

Compound **78** exhibited IC_{50} of 0.01 μM and 0.14 μM on EGF-RTK and KB cell lines, respectively but failed to show activity in tumor xenograft study. While,

compound **79** showed IC_{50} of 0.0064 μM and 0.36 μM on the same cell lines, respectively. Further, modification at C-6 position in compound **78** provided compound **80**. Compound **80** is slightly less potent in comparison to **78** as IC_{50} values of **80** were found 0.027 μM and 0.31 μM on EGF-RTK and KB cell lines, respectively, but showed better activity in tumor xenograft study.

Smaill *et al.* carried out the synthesis and biological activity of C-6 and C-7-substituted quinazolines of prototype **XIII** and **XIV** as shown in Figure 28.[51]

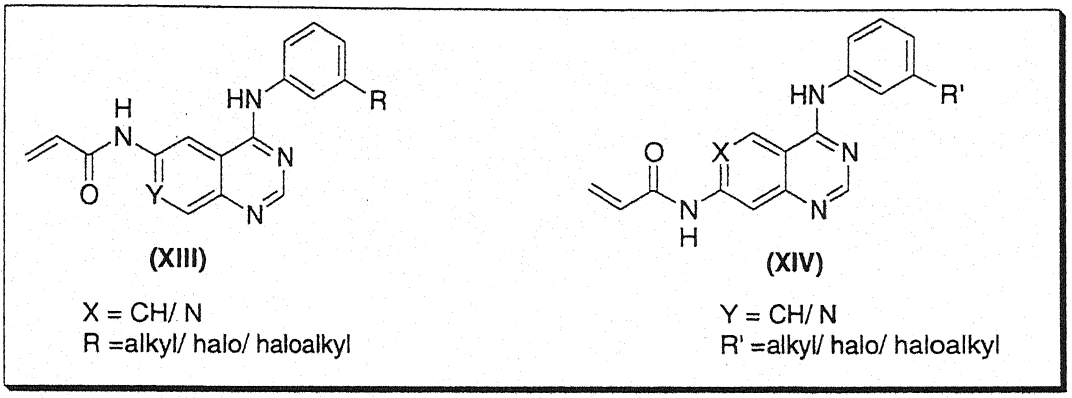


Figure 28: Quinazoline Prototypes **XIII** and **XIV**

The study was based on the 4-(phenylamino)quinazolines **81** and **82**, which are identified as irreversible inhibitors of EGFR as shown in Figure 29.[52] The aim of study was to improve the solubility as well as antitumor activity.

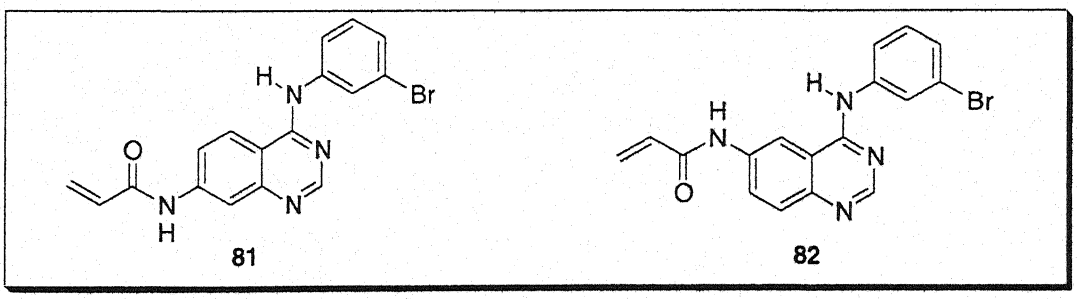


Figure 29: Derivatives of quinazoline (Compound **81-82**)

Modeling data suggested that C-6 and C-7 positions could be substituted with bulky solubilizing functionalities. Acrylamido side chain (Michael acceptor) at C-6 position is optimally placed for reaction with Cys-773 in its most stable binding mode, whereas the acrylamido side chain at C-7 would require some movement in the binding site to allow it to approach close enough to the sulfur for Michael addition to occur, which is also proved by experimental results.

SAR showed that on changing the central chromophore of 7-acrylamide quinazoline (**81**) to pyridopyrimidine leads to the loss of property for irreversible inhibition; while the 6-acrylamide pyridopyrimidine retained the irreversible inhibition activity. However, quinazoline derivatives **81**, **82**, **83** and **84** showed IC_{50} of 0.45, 0.70, 0.42 and 0.75 nM on EGFR isolated enzyme inhibition, respectively, whereas the pyridopyrimidine derivatives **85**, **86** and **87** showed IC_{50} of 0.91, 0.72 and 0.77 nM, respectively. These compounds were also tested for *in vivo* activity against the A431 epidermoid and H125 non-small-cell lung cancer human tumor xenograft models. *In vivo* studies showed significant tumor growth inhibition and acceptable therapeutic indices. The net tumor cell kill values were around –0.5 to +0.5 log unit. Structures of the compounds are shown in Figure 30.

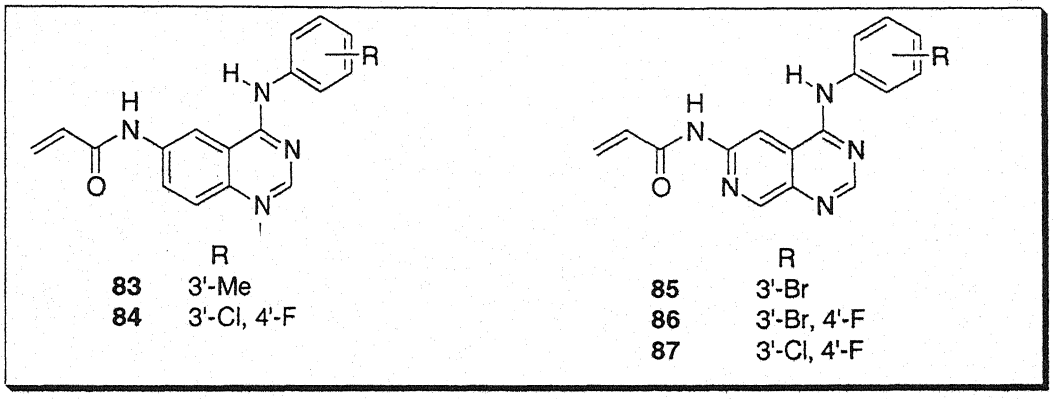


Figure 30: Derivatives of quinazoline (Compound **83-87**)

Smaill *et al.* synthesized acrylamide-substituted 4-anilinoquinazolines and 4-anilino[*d*]pyrimidines derivatives of prototype **XV**.[53] This prototype is related to compounds **88** and **89**, which are selectively potent irreversible inhibitors of tyrosine kinase of *erb*B family.[51] Substitution was carried out at C-7 position with solubilizing cationic side chains (alkylamine and alkoxyamine). These compounds showed better aqueous solubility, potency and *in vivo* antitumor activity. Substitution of C-4' of the aniline ring with cationic side chain caused loss of binding affinity in ATP binding domain of EGFR. The acrylamide analogues with solubilizing cationic side chain provided the irreversible inhibitors. While, the cationic unsaturated side chain at C-7 position in case of pyrido[3,2-*d*]-pyrimidines provided highly potent compound but these compounds showed several problems related to metabolism, transport, permeability and stability.

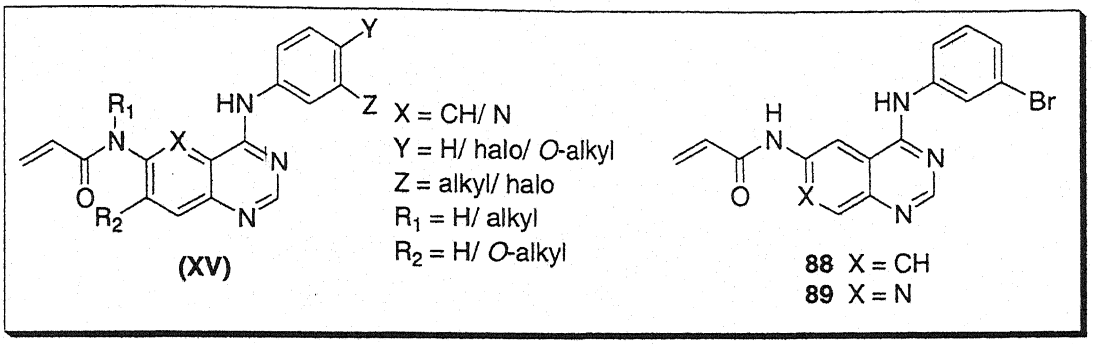


Figure 31: Quinazoline prototype **XV** and derivatives (Compound **88-89**)

Based on this study, a series of "oxygen linked" quinazoline (compound **90**) was synthesized. Compound **90** (CI-1033) exhibited IC_{50} of 1.5 nM in isolated EGFR enzyme inhibition and IC_{50} of 7.4 and 9.0 nM in autophosphorylation assay against EGFR and *erb*B2, respectively.

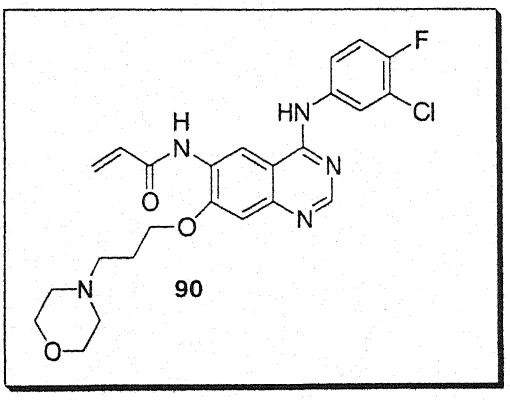


Figure 32: Derivative of quinazoline (Compound **90**)

Compound **90** also showed superior *in vivo* antitumor activities, when compared to earlier nonsolubilized analogues. As discussed above, CI-1033 (compound **90**), having 6-acrylamide group, is an irreversible inhibitor of both EGFR and erbB2 autophosphorylation and showed more activity in comparison to reversible inhibitors. Based on this study, Smaill *et al.* synthesized several 4-anilinoquinazoline and 4-anilnopyrido[3,4-*d*]pyrimidine analogues of prototype **XVI**,[54] in which different substituted Michael acceptor were placed at C-6 position with different solubility enhancing group as shown in Figure 33.

SAR studies showed that *N*-methyl at C-6 position (**91**) possessed irreversible inhibition with high potency while the larger group decreased the activity. Substitution at α-allylic position is not tolerable. Substitution at β-position in acrylamide by electron withdrawing groups increase the electrophilicity of the double

bond of the Michael acceptor and resulted in fully irreversible compounds but a balance is required in reactivity of Michael acceptor and steric bulk at this position. A little difference of activity was observed in between the quinazoline and pyrido[3,4-*d*]-pyrimidine chromophores. Compounds bearing non-acrylamide Michael acceptor at C-6 position (like sulfonamide) showed potent and irreversible inhibition of EGFR but these compounds were unstable in the biological system.

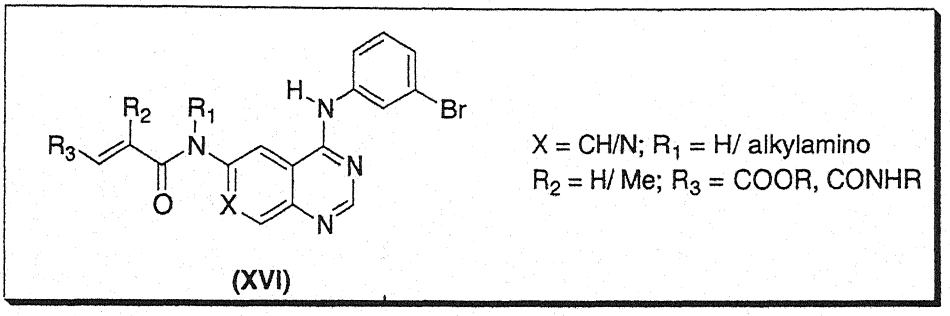


Figure 33: Quinazoline prototype **XVI**

Compounds **91** and **92** were found most active with IC_{50} of 0.17 nM and 0.76 nM against the EGFR enzyme, respectively as shown in Figure 34. But both the compounds were ineffective against the A431, H125 and MCF-7 xenografts.

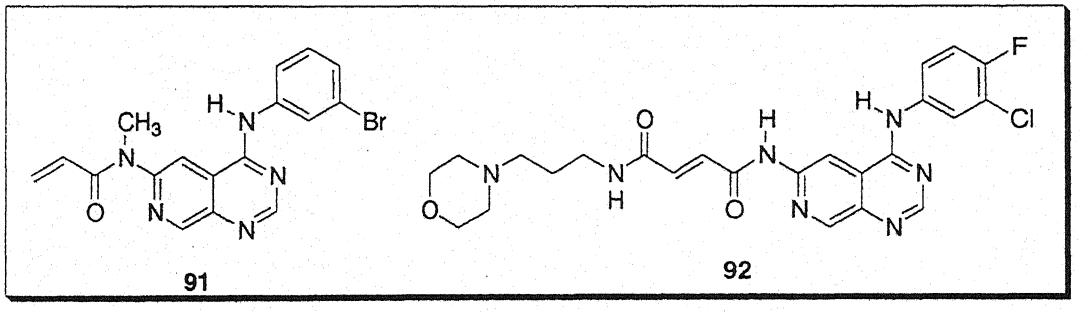


Figure 34: Derivatives of quinazoline (Compounds **91** and **92**)

Based on the 4-(anilino)quinazoline core structure, irreversible inhibitors of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER-2), various derivatives of prototype **XVII** have been synthesized by the Tsou *et al.* as shown in Figure 35.[55]

The designing of the derivatives was based on the two earlier active compounds **82** and **93**.[56,57] These compounds (**82** and **93**) exhibit poor bioavailibility due to low solubility under physiological condition. In order to improve the water solubility, the water solubilzing groups (dialkylamino) were attached directly on the Michael acceptor (crotonamide group), which is available at position C-6.

Dialkylamino group exists as protonated form and exerts the inductive effect on Michael addition of sulfhydryl group of Cys 773. Compound **94** (Figure 36) showed better oral antitumor activity in comparison to compounds **82** and **93**.

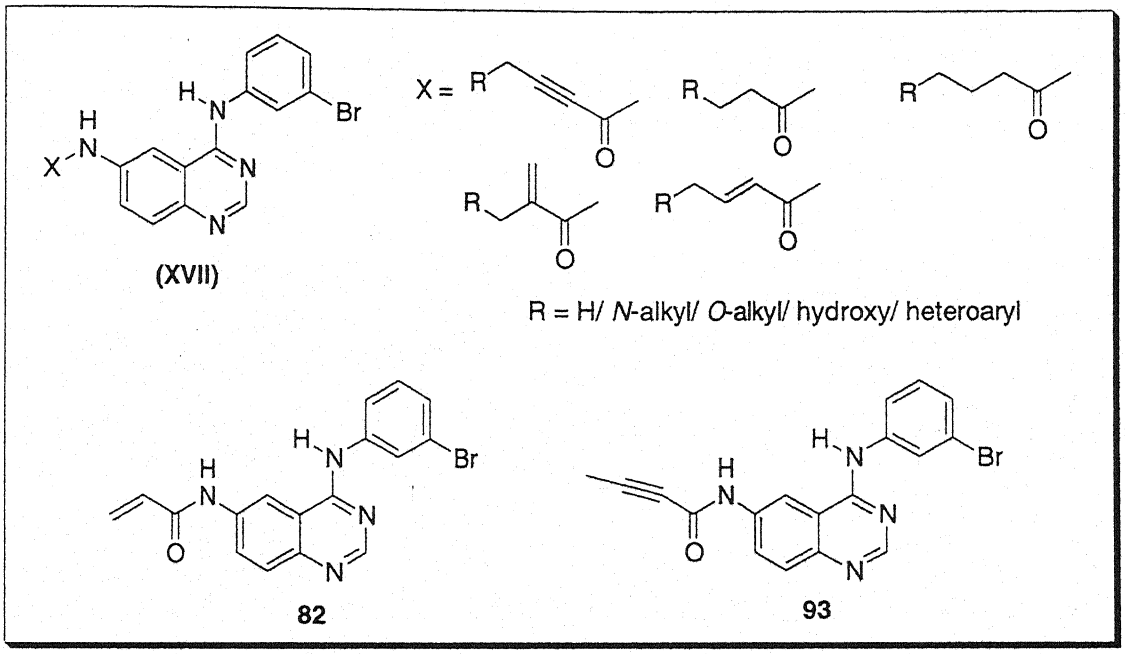


Figure 35: Quinazoline prototype **XVII** and derivatives (Compound **82-93**)

Compound **94** is highly potent in inhibiting EGFR and HER-2 kinases receptors with IC_{50} of 0.011 μM and 0.301 μM, respectively. However, compound **94** was less potent in inhibiting KDR, EcK, Mek/ErK, PDFGR and VEGF kinases as IC_{50} value was found > 40 μM.

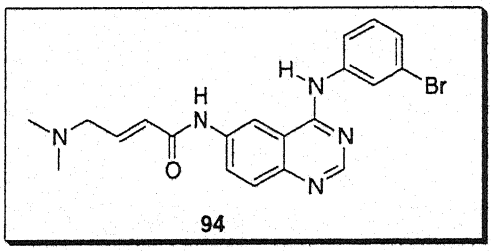


Figure 36: Derivative of quinazoline (Compounds **94**)

Compound **95** is a potent inhibitor of EGFR-TK but undergoes rapid metabolism on oral dosing.[58,44] To increase its stability, methyl group was replaced by the chloro and fluoro at C-3' and C-4', respectively (compound **77**). Although potency of compound **77** was decreased in comparison to compound **95** but it showed better *in vivo* profile and improved efficacy. Barker *et al.* carried further attempts to increase *in vivo*

activity by modifying the methoxy group in compound **77** and synthesized the compounds of prototype **XVIII** as shown in Figure 35.[59]

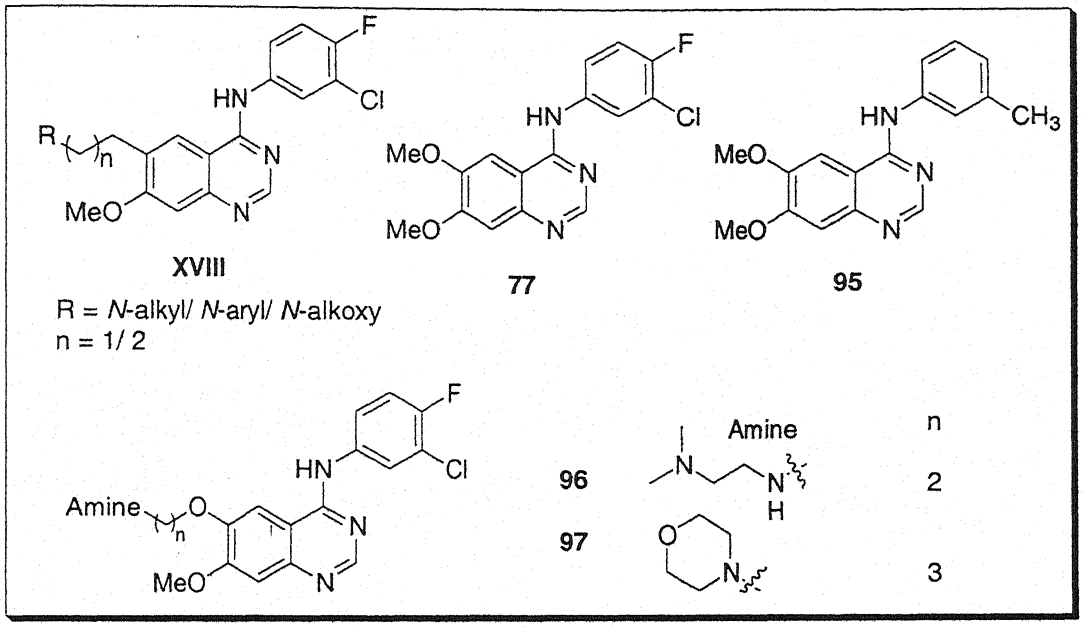


Figure 37: Quinazoline prototype **XVIII** and derivatives (Compound **77, 95-97**)

SAR studies exhibited that morpholino derivatives possessed high blood concentration after 6h and also sustained at 24h but the compound with 2-hydroxy substituent at C-6 position showed less blood concentration. Compound **96** was identified as a most potent enzyme inhibitor with IC_{50} value of 2 nM. In EGF-stimulated cell proliferation test, compound **97** was found as a most potent inhibitor of tumor cell growth with IC_{50} of 80 nM. Compound **97** inhibits the growth of a broad range of human solid tumor xenografts, which was later launched in the market as Gefitinib (Irresa).

Cockreill *et al.* has found compound **98** (4557W) acts as a potent inhibitor of c-erb-2 and EGFr.[60] The binding hypothesis has shown that benzyloxyaniline group, accommodated in the back of hydrophobic pocket with 6,7-dimethoxy groups, is pointing towards the tip of ATP binding cleft. A conformational restriction was carried out at the aniline fragment by placing different bicyclic ring (such as substituted indole, indazole, imidazole and benzotriazole) in the quinazoline and pyrido[3,4-*d*] pyrimidine ring systems of prototype **XIX**, to study the effect of hydrophobic binding interaction as shown in Figure 38.[61]

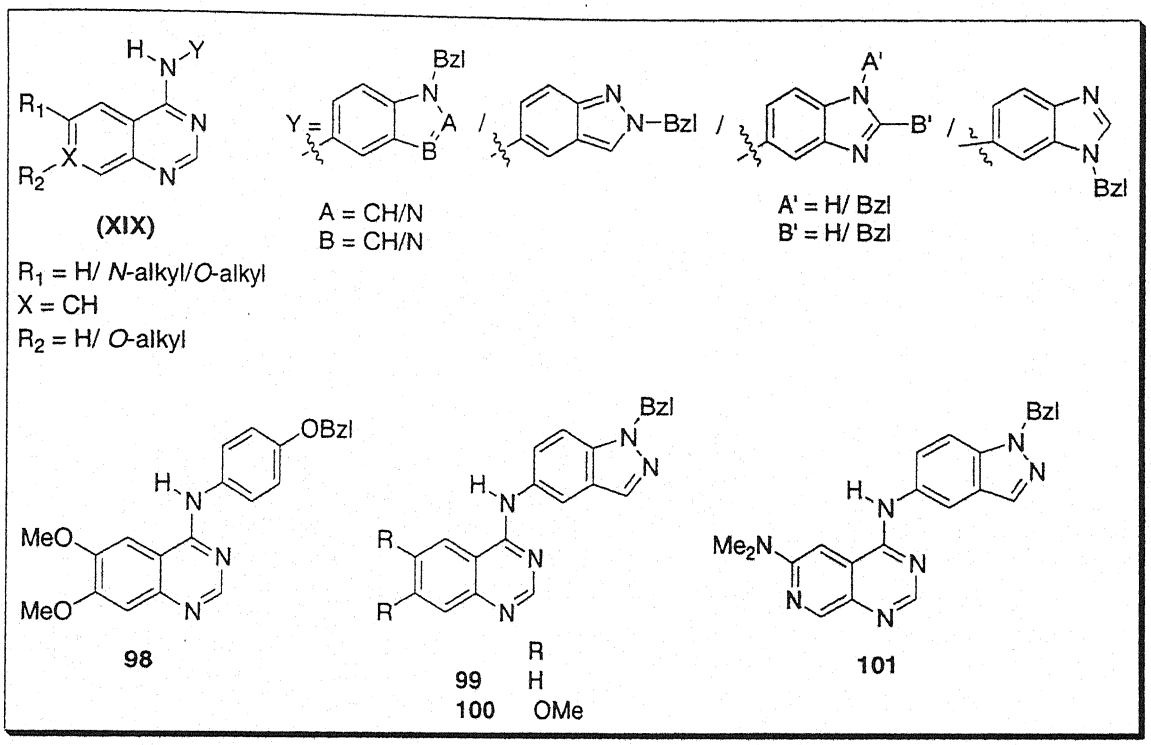


Figure 38: Quinazoline prototype **XIX** and derivatives (Compound **98-101**)

The compounds **99**, **100** and **101** were synthesized and evaluated for their anticancer activity and showed IC_{50} of 0.01, 0.001 and 0.027 μM, respectively against c-erB-2 inhibition (enzyme potency). Amongst them, compound **99** (GW974) showed the selectivity for EGFR subfamily. Compound **101** also exhibited the activity against CaLu3, HN5 and BT474 cells. Compound **101** showed 24% bioavailability in comparison to 7% of corresponding quinazoline analogue **100.**

Albuschat *et al.* synthesized various anilinoquinazoline derivatives of prototype **XX** as **102**, **103** and **104**.[62] The study was based on the EGFR tyrosine kinase inhibitory activity of levendustin (**102**) and salicylanilides derivatives **103** and **104** as shown in Figure 39.[63]

Based on the bioisosteric relation between salicylic and quinazoline group, quinazoline derivatives **105** and **106** were synthesized. These anilinoquinazoline derivatives were further tried to convert into irreversible EGFR inhibitors, which contain the Michael acceptor position similar to CI-1033 (**90**).

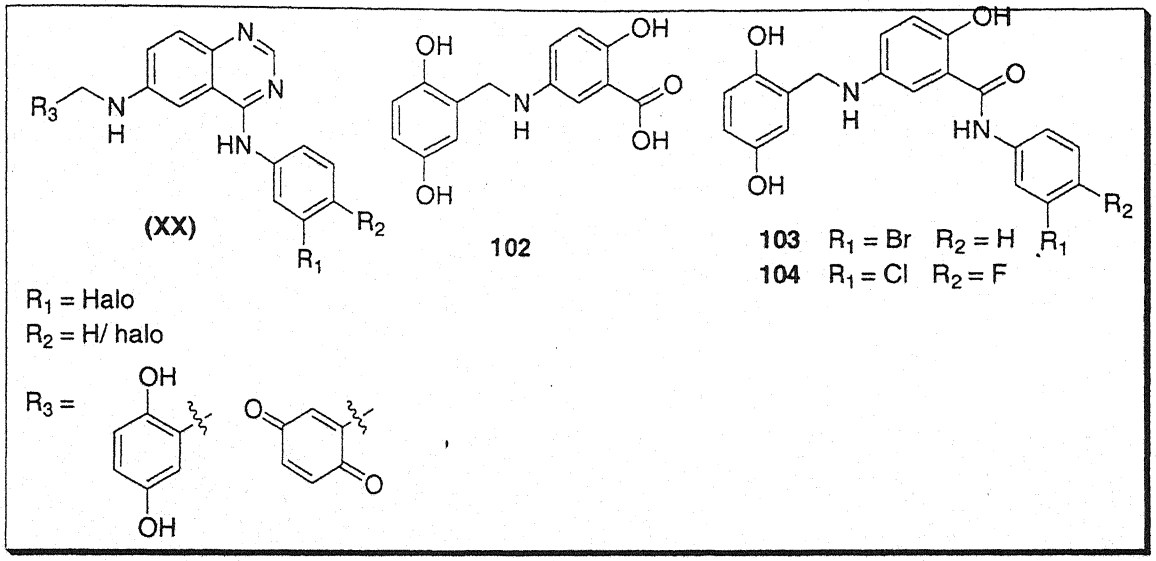


Figure 39: Quinazoline prototype **XX**, levendustin (compound **102**) and salicylanilides derivatives (compounds **103** and **104**)

Compounds **105** and **106** (Figure 40) showed observable inhibitory effects on U87MG, A172 T98G cell lines and potent EGFR tyrosine kinase inhibitory activity with IC_{50} in the range of 0.1-1μM. Compound **106** exhibited high activity against CCRF-CEM (GI_{50} = 0.04 μM, TGI = 0.09 μM) and A498 (GI_{50} = 0.23 μM). The LC_{50} value on leukemia cancer cell line was observed 33.7-98.7 μM with compound **106**. Both the compounds **105** and **106** showed LC_{50} value of 51.5-98.7 μM in NSCLC, 61.4-74.1 μM in melanoma and 42.4 & 40.8 μM in renal A498 cancer cell lines, respectively.

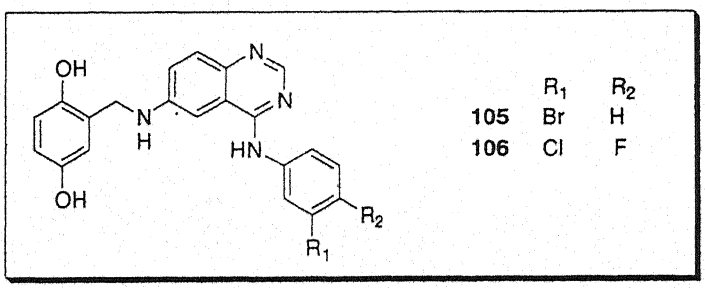


Figure 40: Derivatives of quinazoline (Compounds **105-106**)

Jin *et al.* synthesized a number of 5–sustituted-4-hydroxy-8-nitroquinazoline derivatives of prototype **XXI** that caused the inhibition of both EGFR and ErbB-2 tyrosine kinases as shown in Figure 41.[64] The study was based on molecule B-17 (**107**), an inhibitor of ErbB-2 TK. [65] The nitro group has been substituted at C-8 position of the 4-hydroxyquinazoline (**108** and **109**).

SAR studies indicated that aniline moiety is essential at C-5 position. Further substitution at C-4' position in aniline ring with electron donating group exhibited a positive remarkable effect for example, 4'-methoxy substituted compound (**108**) showed best dual inhibition of both EGFR and ErbB-2 signaling with $IC_{50} \leq 0.01$ and 13.0 μM, while large benzyloxy substituted compound (**109**) caused more inhibition of ErbB-2 signaling with IC_{50} of 7.0 μM. Compounds **108** and **109** showed promising antiproliferative effect against the EGFR and ErbB-2-overexpressing tumor cell lines.

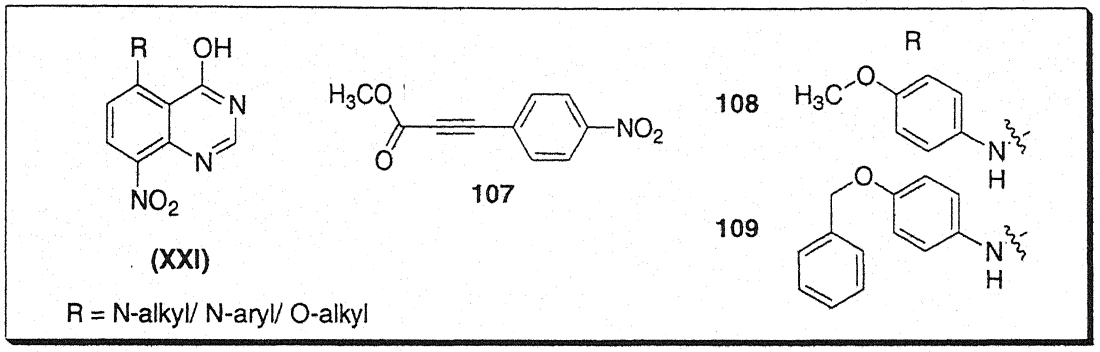


Figure 41: Quinazoline prototype **XXI**, B-17 (**107**) and derivatives (compounds **103** and **104**)

Ballard *et al.* synthesized various 5-substituted 4-aminoquinoline derivatives of prototype **XXII** as ErbB2 receptor tyrosine kinase inhibitor as shown in Figure 42 .[66] The study was based on the lead compound **110**, which has IC_{50} of 0.0056 μM for inhibition of ErbB2 kinase enzyme.[67]

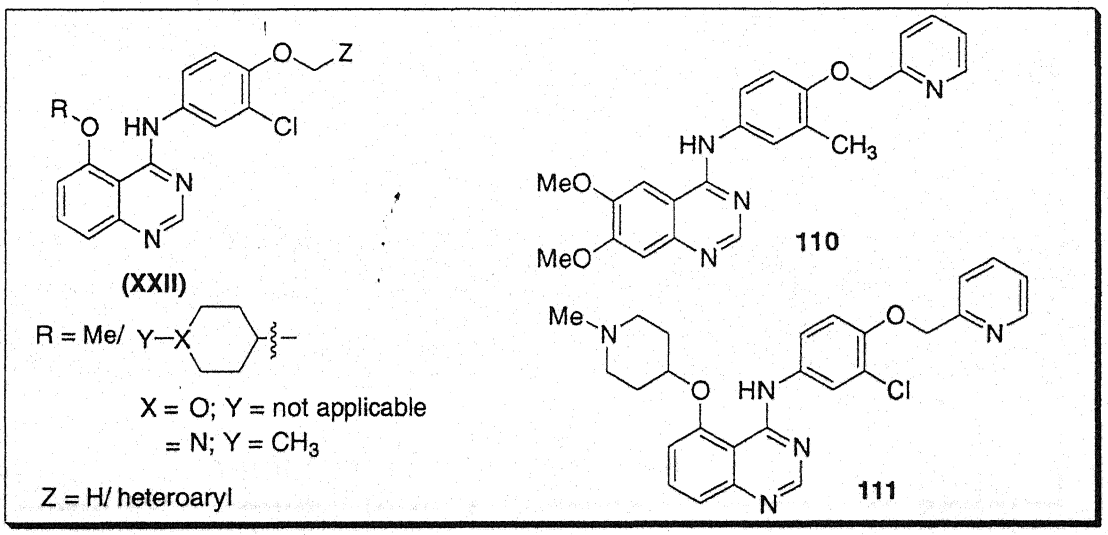


Figure 42: Quinazoline prototype **XXII** and derivatives (compounds **110** and **111**)

Aniline side chain of compound **110** was assumed to be interacting with the selective pocket at erbB2 active site. Based on the homology model and work carried out on inhibitors of c-Src, substituent was shifted from C-6 position to C-5 in quinazoline, which can now occupy kinase sugar pocket and improve the affinity towards ErbB2. SAR studies presented that the transferring of substituent from C-6 position to C-5, introduction of a cyclic amine and changing the *meta*-substituent in the extended aniline from methyl to chlorine, significantly increased the affinity for both erbB2 and EGFR kinases. Further, upon removal of C-7 methoxy group provided the selectivity for erbB2. Compound **111**, however identified as potent compound, which showed IC_{50} < 0.002, 2.4, 0.027 and 0.46 μM on erbB2, EGFR, BT474C and KB cell line, respectively.

Ballard *et al.* realized that little work is done at C-5 in anilinoquinazolines as EGFR inhibitors and synthesized various molecules of prototype **XXIII** as shown in Figure 43.[67] Simple substitutions at C-5 position in quinazoline decreased the activity and generally, this position is kept unsubstituted.

On examining the overlay of ATP with the known binding mode of anilinoquinazoline, it was predicted that potent inhibitor could be obtained on placing the appropriate substituent at C-5 position, which can utilize the ribose-binding pocket.

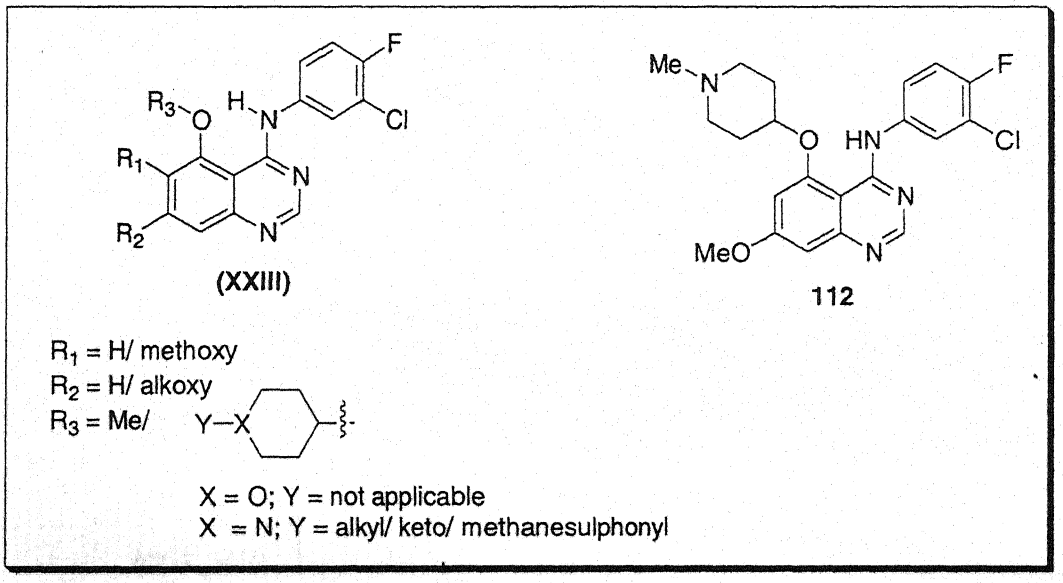


Figure 43: Quinazoline prototype **XXIII** and derivative (compound **112**)

SAR studies showed that at C-5 position, basic cyclic group exhibited potent enzyme inhibition, which may be due to electrostatic complementaries with the cationic side chain. Cyclic group, without this charge, were lacking in the activity. Similarly, open chain analogue also exhibited less activity. Moving the methoxy from C-7 to C-6 position decreased the activity. Compound **112** showed IC_{50} of 21 nM on EGFR and 87 nM on EGF-driven KB cell line.

1.4.2 VEGFR Inhibitors:

Hennequin *et al.* has developed substituted 4-anilinoquinazolines and related compounds of prototype **XXIV** as VEGFR inhibitors as shown in Figure 44.[68]

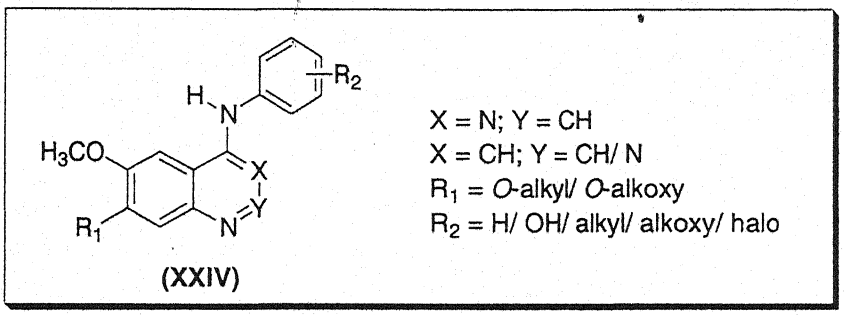


Figure 44: Quinazoline prototype **XXIV**

SAR studies showed that small lipophilic substituents are preferred at C-4' position like halogen or methyl, whereas the small substituents like hydrogen or fluorine are preferred at C-2' position. Introduction of the hydroxy group in aniline produced the highly potent compounds (e.g. **113-116**) as shown in Figure 45. Further, modifications at C-7 position also provided potent compounds.

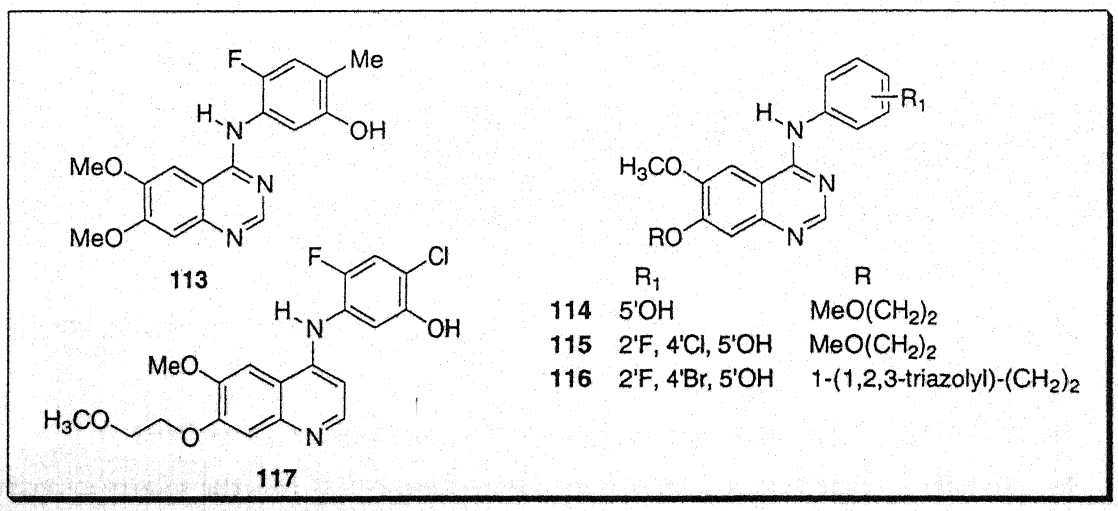


Figure 45: Derivatives of quinazoline (Compounds **113-117**)

Two endothelium associated with high affinity RTK's for VEGF have been identified, the fms-like tyrosine kinase receptor, Flt, and the kinase insert domain-containing receptor, KDR (also referred to as Flk-1 in mice). Compound **113** and **114** showed IC_{50} value of 0.003 μM on Flt cell line. Compound **115** exhibited IC_{50} value of < 0.002 μM on Flt and KDR cell line and 0.004 μM on VEGFR cell line. Compound **117** showed IC_{50} value of 0.003 and 0.002 μM on Flt and KDR cell line.

Hennequin *et al.* synthesized various quinazoline derivatives of prototype **XXV** as shown in Figure 46,[69] based on the earlier observations that aniline ring consisting of small lipophilic electron deficient substituent at the C-2' position along with larger lipophilic electron withdrawing atom at C-4' position showed potent activity.

SAR studies exhibited that the replacement of the C-4' hydrophilic electron withdrawing substituent by more lipophilic group have increased the C-π character and leads to improvement in both Flt-1 and KDR inhibition. Introduction of fluorine atom at C-6' position increased the selectivity for the Flt-1 over KDR.

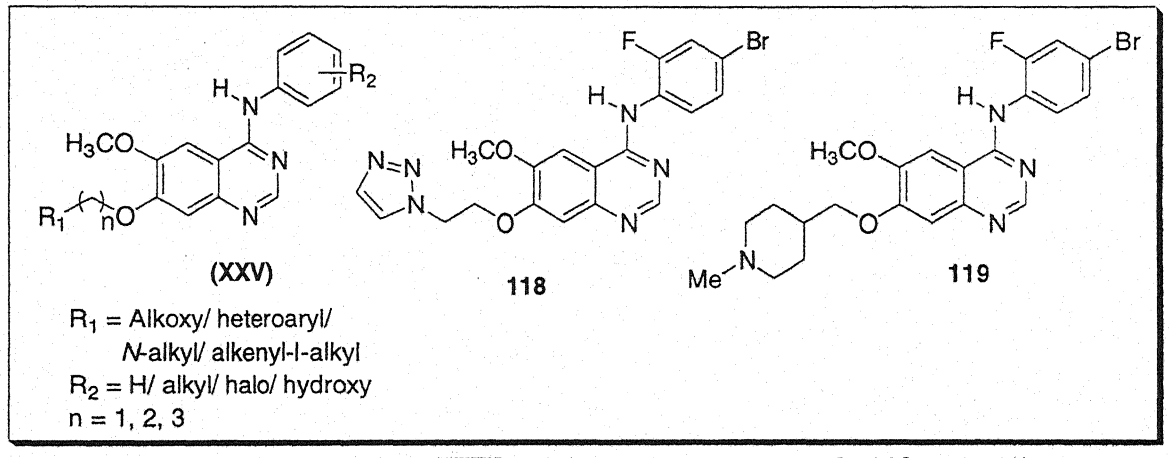


Figure 46: Quinazoline prototype **XXV** and derivatives (compounds **118** and **119**)

This pattern earlier leads to the active compound **118** (ZD4190) in which C-7 chain was neutral.[41] Replacement of this neutral side chain by the basic *N*-methylpiperidine (compound **119**) leads to excellent level of KDR inhibition with improved physicochemical properties and plasma half-life. Compound **119** showed IC_{50} of 0.04 μM and 1.6 μM on KDR and Flt-1 cell lines, respectively.

Wissner *et al.* synthesized quinazolines of prototype **XXVI** with different reactive groups attached at C-4-position and studied the covalent binding inhibition of VEGFR-2 as shown in Figure 47.[70]

Compound **120** showed IC_{50} of 5.1 nM in presence of 10 μM concentration of ATP. In presence of 100 μM of glutathione and 10 μM concentration of ATP, IC_{50} of compound **120** was found to be 6.1 nM. Compound **120** was also tested in other kinases like AKT, BTK, CDK4, EGFR, GSK, IGFR, IKK, ITK etc and in these assays, IC_{50} varied between 1.8 to >117 μM. These activity results prompted its *in vivo* evaluation. The oral administration of **120** resulted significant reduction in the tumor growth. Efficacy of the **120** is comparable to Bevacizumab, which is a neutralizing antibody to VEGF.

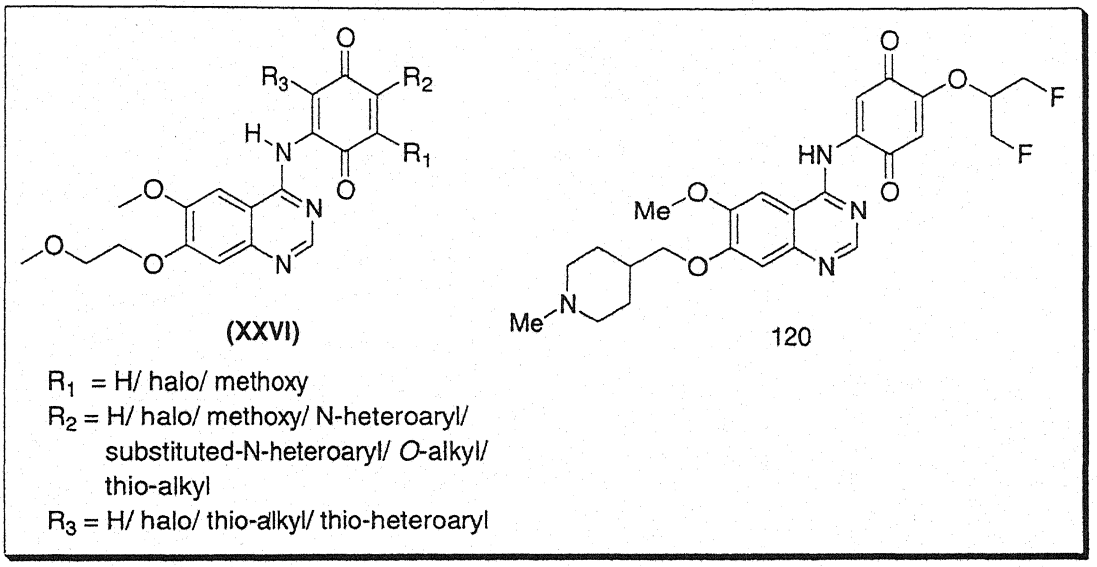


Figure 47: Quinazoline prototype **XXVI** and derivative (compound **120**)

1.4.3 PDGFR Inhibitors

Matsuno *et al.* carried out synthesis of various quinazolines derivatives of prototype **XXVII** as shown in Figure 48.[71] This study was based on the potent anticancer molecule KN1022 (**158**), in which the modifications were carried at 4-nitrophenylurea moiety. These molecules were evaluated for the inhibition of β-PDGFR phosphorylation.[72]

SAR studies in the series exhibited that position and nature of the substituents on the phenyl ring attached to urea moiety have a substantial influence on the inhibitory activity. Substitution at C-4' in phenyl ring was the most favorable, while the activity was reduced upon incorporating the substitutions at C-3' and C-2' positions. Bulky hydrophobic substituents at C-4' position increased the activity. Small substituent like methyl at bulky phenoxy group (compound **126**) is also exhibited potent activity.

The introduction of hydrophilic substituent at C-4' is unfavorable. Thiourea derivative also showed inhibitory activity on β-PDGFR phosphorylation but lesser than ureas. However, compounds **122-126** exhibited IC_{50} of 1.10, 0.53, 0.23, 0.08 and 0.02 μmol/L in comparison to 0.70 μmol/L of **121**, for the inhibition of β-PDGFR phosphorylation.

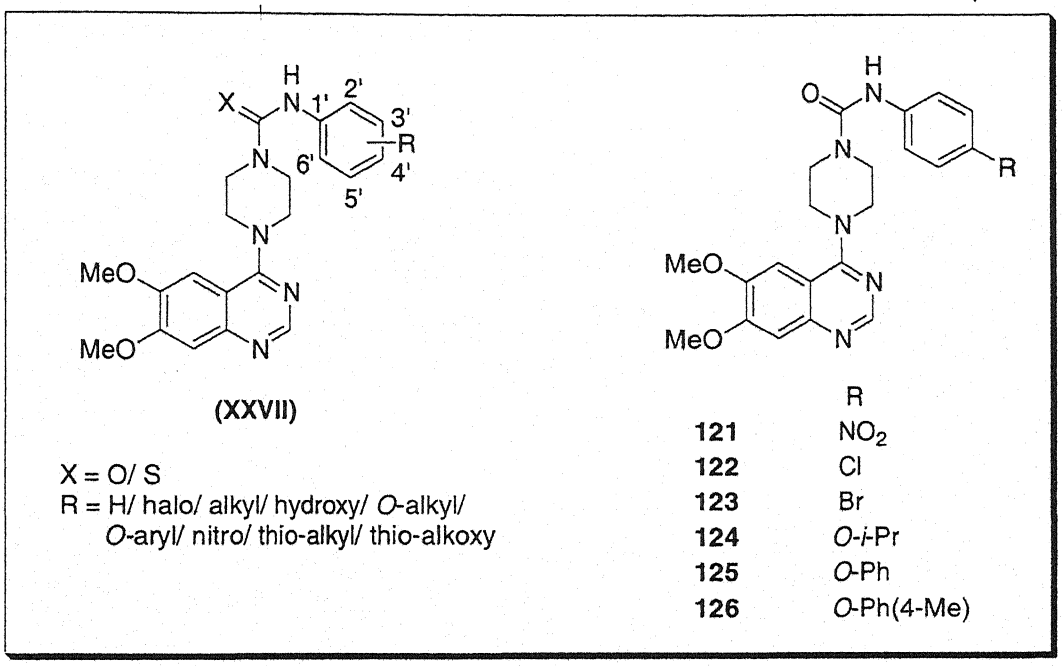


Figure 48: Quinazoline prototype **XXVII** and derivatives (compounds **121-126**)

Compounds **122**, **123** and **125** were also evaluated for the inhibition of SMC proliferation induced by PDGF-BB. Compounds **122**, **123** and **125** showed good oral absorption and high plasma drug concentration and significant inhibition of neointima formation in the range of 24-38%, which can be useful for the treatment of the atherosclerosis.

Pandey *et al.* synthesized various β-PDGFR phosphorylation inhibitors of prototype **XXVIII** as shown in Figure 49.[73] The study was based on the existing 4-piperazinylquinazoline derivative CT52923 (compound **127**)[74,75] The aim was to increase the potency, appropriate kinase specificity including high oral bioavailability and long plasma half-life.

Various modifications were carried out at A and D ring. In the D ring, 4-isopropoxy or cyano substituent provided the compounds with maximum metabolic stability, oral bioavailibility and plasma half-life. In the A ring, the presence of

propoxy basic side chain at C-7 position leads to improved aqueous stability, bioavailability, improved inhibitory activity, reduced protein binding and good potency. Simultaneously, upon replacement of urea linkage by isosteric thiourea unit showed good inhibitor activity. Compound **128** showed IC_{50} value of 26 and 36 nM on MG63 cell line in the absence and presence of the plasma, while IC_{50} value is 200 nM in Chinese hamster ovary cell line. Compound **128** also inhibits autophosphorylation of a constitutively activated Flt3/ITD mutant expressed in hematopoietic cells or AML cell lines with IC_{50} value of 30-100 nM.

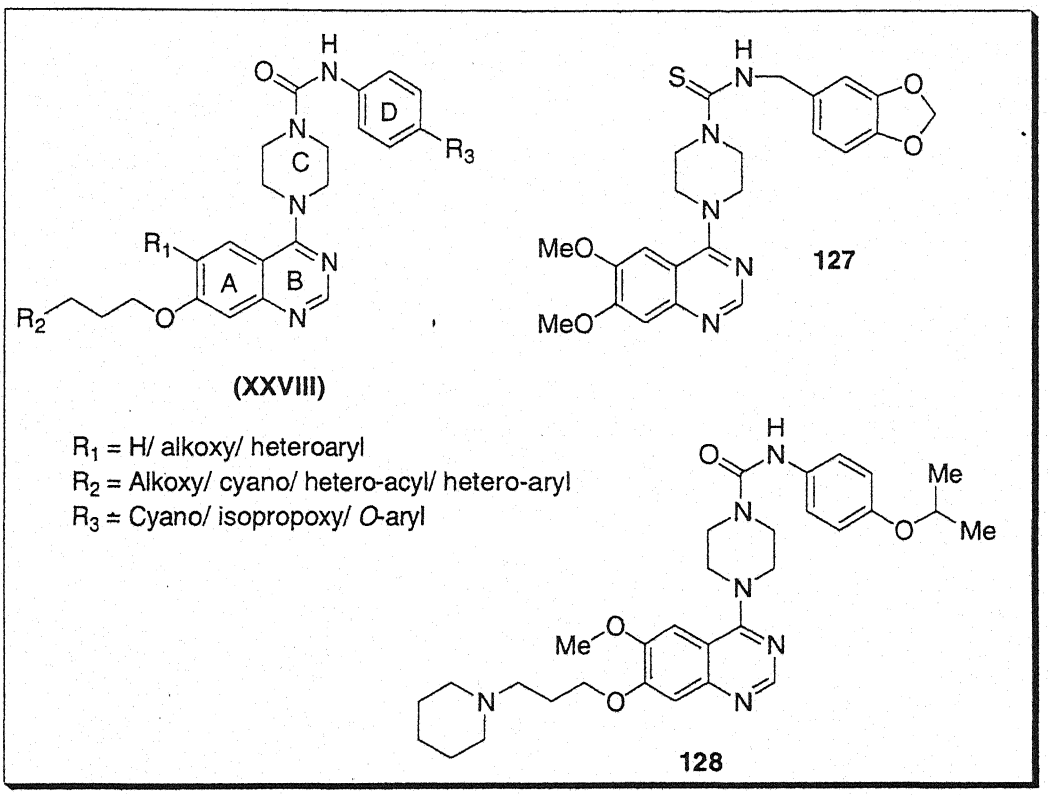


Figure 49: Quinazoline prototype **XXVIII** and derivatives (compounds **127** and **128**)

Matsuno *et al.* carried out the synthesis of similar quinazoline derivatives of prototype **XXIX** and studied SAR for inhibition of *β*-PDGFR phosphorylation.[76] The designing of the molecules was based on the molecule KN1022 (**121**), which is a potent inhibitor PDGFR phosphorylation as shown in Figure 50.

SAR studies indicated that the change of the linker between the phenyl ring and the (thio)urea moiety has a major effect on the potency. In the urea derivatives, insertion and extension of the methylene chain reduced the activity and which is opposite in case of benzylthiourea derivatives. Benzylthiourea with relatively small substituents on C-4' such as compounds **129-131** and 3,4-methylenedioxy group such

as compound KN734 (**132**) were found to be most suitable. Upon replacement of the phenyl ring with other heterocyclic ring system, improvement in the aqueous solubility has been observed. Thienyl analogue (**135**) showed activity similar to **121**. Compound **133** possessing 3-pyridine ring with methylene bridge and furfuryl ring derivative (**134**) showed high aqueous solubility.

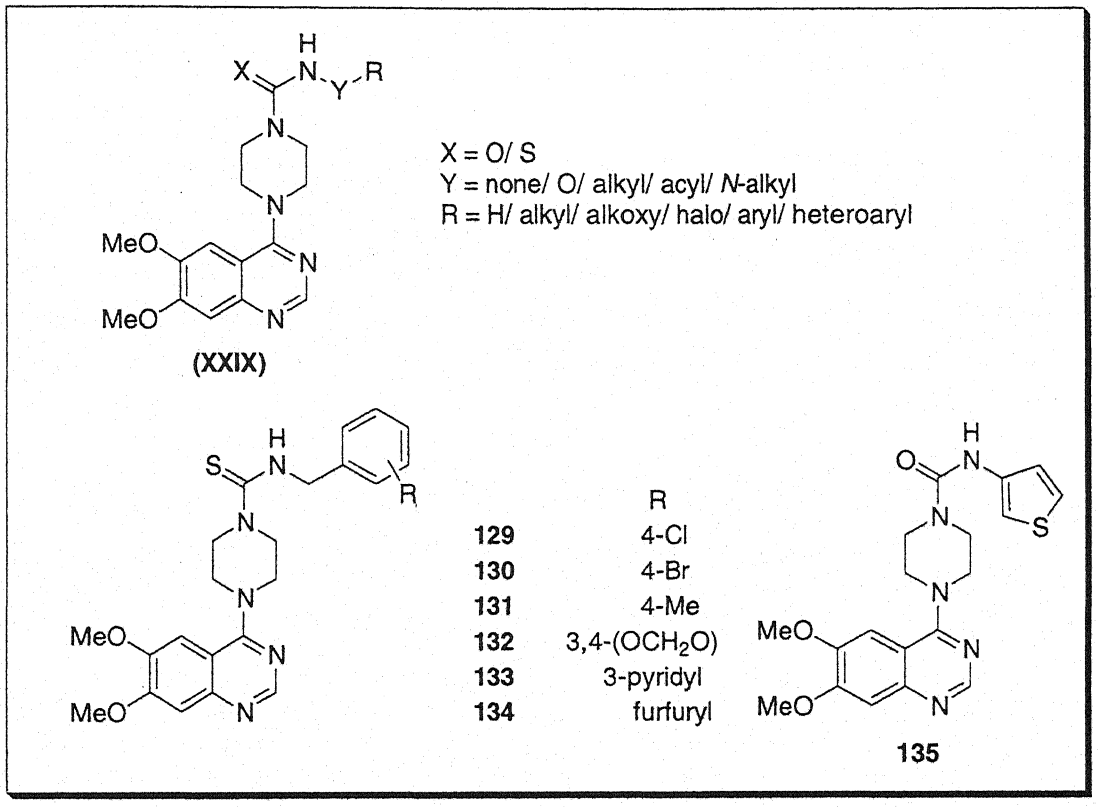


Figure 50: Quinazoline prototype **XXIX** and derivatives (compounds **129-135**

It was observed that acidic hydrogen on the (thio)urea moiety is essential for interaction with PDGFR. Modification in the piperazine ring had no positive influence on the activity. Compounds **129**, **130**, **131** and **132** exhibited IC_{50} value of 0.07, 0.03, 0.03 and 0.09 μmol/L, respectively, on β-PDGFR inhibition. Compounds **132**, **134** and **135**, however, showed good oral absorption and high plasma concentration. These compounds also cause inhibition of neointima formation. These compounds may represent a new approach for atherosclerosis treatment.

Based on the previously known PDGFR phosphorylation inhibitor, active molecules KN1022 (**121**) and KN734 (**132**) of quinazoline derivative, SAR studies was carried out by Matsuno *et al.* and various compounds of prototype **XXX-XXXII** were designed and synthesized as shown in Figure 51.[77]

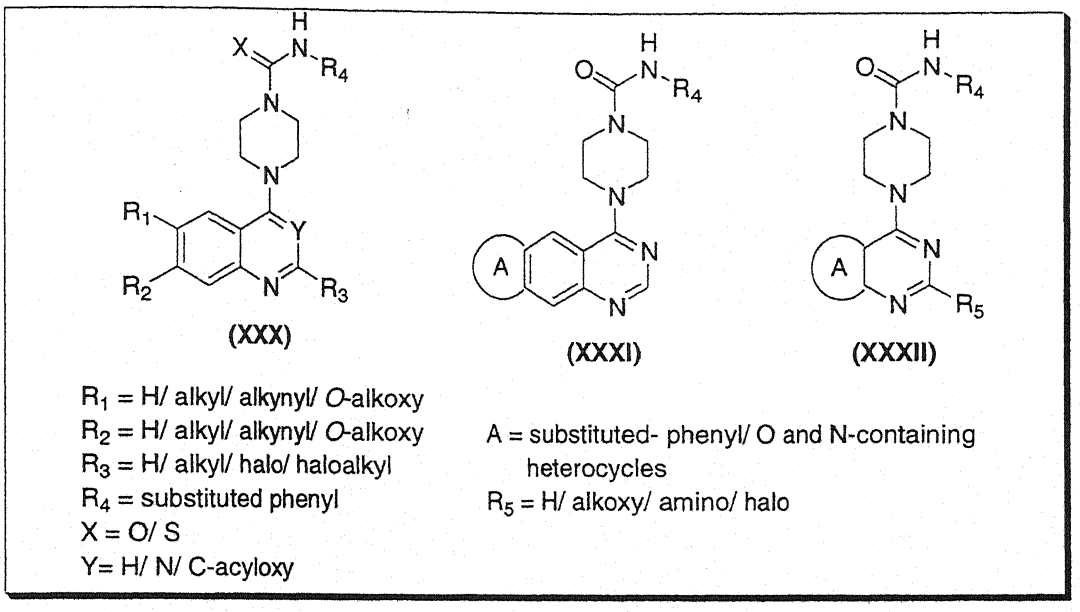
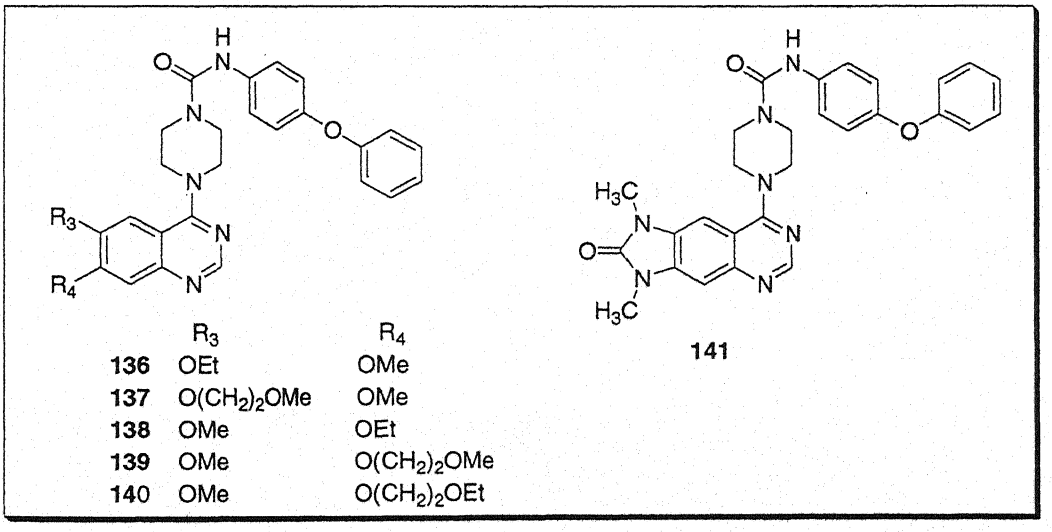


Figure 51: Quinazoline prototype **XXX- XXXII**

SAR studies of the series resulted that the amongst C-6 and C-7-dialkoxy substituents, ethoxy analogue showed potent activity, which includes the compounds **136-140**. Amongst tricyclic quinazoline 2-oxoimidazo[4,5-*g*]quinazoline analogue, compound **141** exhibited potent activity as shown in Figure 52.



	R_3	R_4
136	OEt	OMe
137	$O(CH_2)_2OMe$	OMe
138	OMe	OEt
139	OMe	$O(CH_2)_2OMe$
140	OMe	$O(CH_2)_2OEt$

Figure 52: Derivatives of quinazoline (Compounds **136-141**)

The exchange of the quinazoline ring with other heterocyclic ring furnished compounds pyrazolo[3,4-*d*]pyrimidine (**142**) and quinoline derivatives (**143**), which exhibited potent activity as shown in Figure 53. However, isoquinoline pyridopyrimidine derivatives are found inactive. Therefore, it was concluded that the

N-1 atom has an important role and replacement by N-7 and N-8 atoms in the parent quinazoline ring has detrimental effects. Metabolic polymorphism was also observed for the 6,7-dimethoxy substituents on the quinazoline ring. The introduction of 3-COOEt group to compound **143** had provided **144**, in which the activity was retained.

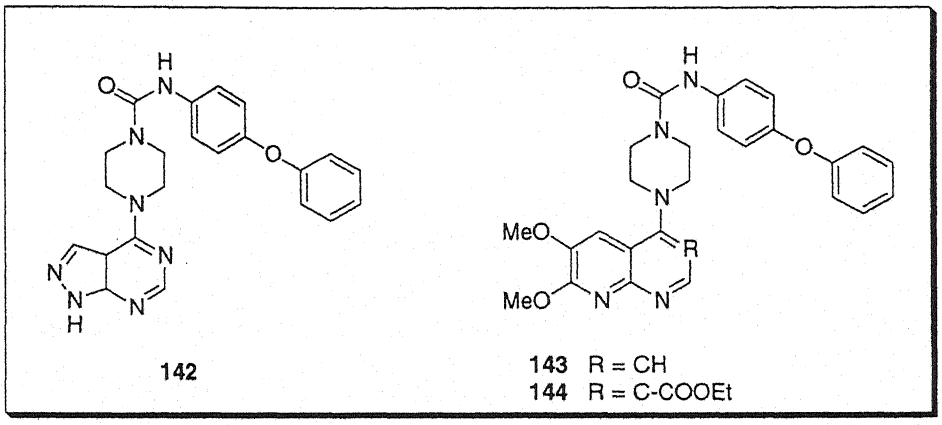


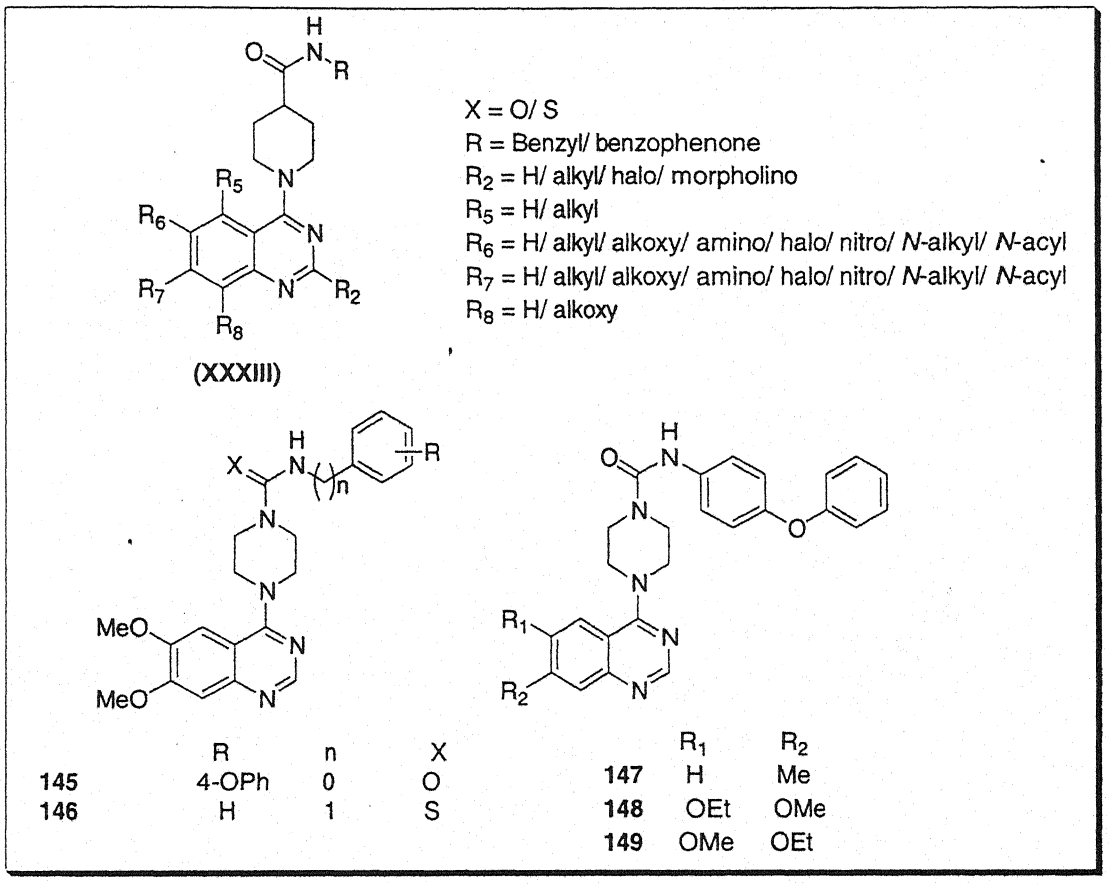
Figure 53: Derivatives of quinazoline (Compounds **142-144**)

Compounds **136-139** showed the IC_{50} of 0.04, 0.02, 0.01 and 0.01 μM against inhibition of β-PDGFR phosphorylation. The tricyclic compound **141** showed IC_{50} of 0.10 μM and pyrazolo[3,4-*d*]pyrimidine derivative **142** exhibited IC_{50} of 0.17 μM. The 6,6-bicyclic-heterocycles **143** and **144** also exhibited IC_{50} of 0.18 μM and 0.09 μM, respectively for inhibition of β-PDGFR phosphorylation.

Matsuno *et al.* studied the effect of combination of substituents on the quinazoline ring and *N*-substituted (thio)urea moiety (prototype **XXXIII**) for several earlier known potent analogues like, KN1022 (**121**) , KN734 (**132**), **145** and **146** as shown in Figure 54.[78]

SAR of these β-PDGFR inhibitors showed that quinazolines compounds without substitution at C-6 and C-7 positions exhibited less order of activity to moderate activity. Quinazoline analogues with mono methoxy group were weaker than dimethoxy analogues. Among the dimethoxy analogues, order of potency is following: C-6 = C-7 >> C-5. Quinazolines possessing C-8 methoxy group had no activity. Polar group and increase in the bulk of halogen at these positions exhibited reduction in activity. In disubstituted (C-6 and C-7 positions) compounds with electron donating group at C-6 exhibited potent activity. Further, addition of group at C-2 and C-8 positions in 6, 7-dimethoxy quinazoline also showed low order of

activity. Compound **147** with C-7 methyl group, however, exhibited the most potent activity with IC_{50} of 0.10 μM/L, while C-6 and C-7 disubstituted quinazoline derivatives compounds **148** and **149** showed equally potent activity and was comparable to dimethoxy compounds **145** and **146**.



	R	n	X
145	4-OPh	0	O
146	H	1	S

	R_1	R_2
147	H	Me
148	OEt	OMe
149	OMe	OEt

Figure 54: Quinazoline prototype **XXXIII** and derivatives (compounds **145-149**)

The 4-[4-*N*-(substituted-thio-carbomyl-1-piperazinyl]-6,7-dimethoxyquinazoline derivatives were earlier reported as potent and selective β-PDGFR inhibitor.[71,76,78,79] Heath *et al.* carried out the optimization study, in order to improve the potency, pharmacokinetic property and synthesized compounds of prototype **XXXIV** as shown in Figure 55.[79]

SAR study was carried out by changing the orientation of aryl/ heteroaryl ring relative to thiourea and changing the distance between thiourea and its aryl group. Piperidine group on C-7 linker gave rise to more potent compounds. While the benzyl thiourea derivatives showed enhanced inhibitory activity in both C-7 ethoxy and C-7 propyloxy linker series. Among the different benzyl thiourea derivatives, compound **150** is found as a most potent derivative, which exhibited selectivity for PDGFR over

Flt-3. In case of heterocyclic ring, the electron-withdrawing groups are preferred. The C-3',4'-disubstituted derivative compound **151** retained the activity. Compound **152,** which is a biaryl derivative, is also a potent compound amongst the aryl thioureas derivatives. Cyanoguanidine derivatives of thiourea series were also synthesized but these compounds have diminished order of activity. Inhibitory activity was checked on the β-PDGFR, c-Kit and Flt-3 and CSF-IR. Compounds **150** (CT53986), **151** (CT53605) and **152** (CT54254) showed IC_{50} value of 61, 129 and 156 nM on MG63 cells respectively.

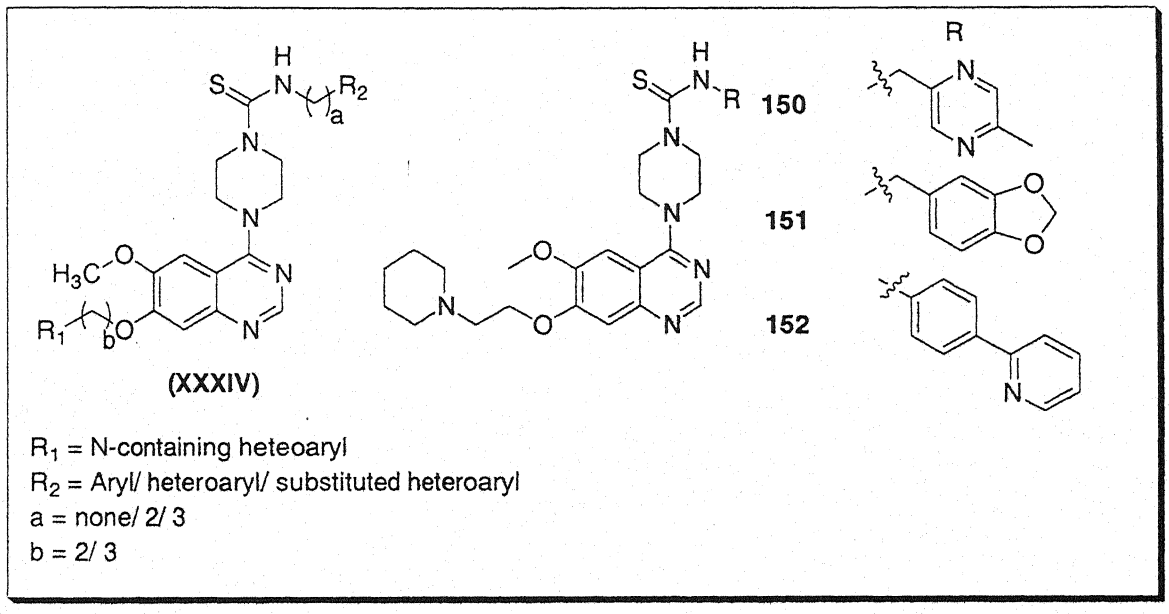


Figure 55: Quinazoline prototype **XXXIV** and derivatives (compounds **150-152**)

1. 5 Quinolines derivatives as anticancer:

Suh *et.* al. synthesized a number of imidazoquinolinedione of prototypes **XXXV** and **XXXVI**, and screened for their anticancer activity as shown in Figure 55.[80] Most of the compounds exhibited potent cytotoxicity against various human cancer cell lines such as A549 (lung), SK-OV-3 (ovarian), SK-MEL-2 (melanoma), XF498 (brain) and HCT-15 (colon). Compound 153 exhibited higher cytotoxicity on HCT-15 as IC50 value was found to be 0.026 μg/mL.

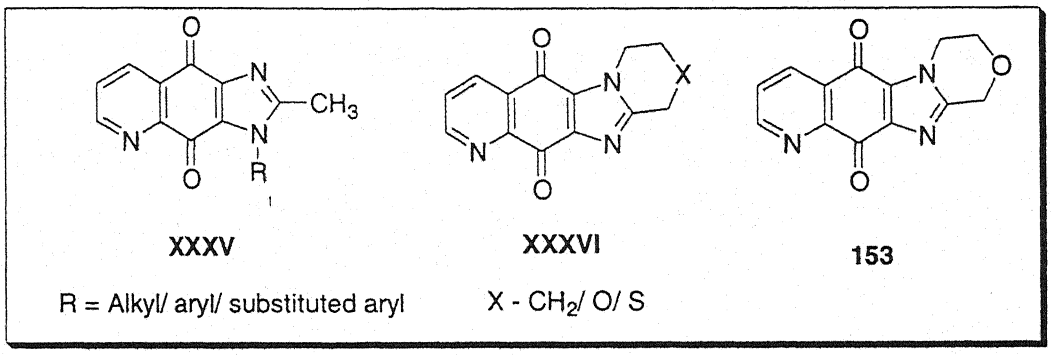


Figure 56: Quinoline prototypes **XXXV**, **XXXVI** and derivative (compound **153**)

A number of hypoxic cell cytotoxins have been designed in order to take a therapeutic advantage of hypoxia. Ortega *et. al.* have already reported that 2-quinolinecarbonitrile-1,4-di-*N*-oxides bearing 3-dimethyl-amino-1-propylamino group to be very potent and selective cytotoxins.[81] With the aim of further improving the hypoxia selective cytotoxicity of 2-quinolinecarbonitrile-1,4-di-*N*-oxides, a number of quinoline derivatives of prototype **XXXVII** was prepared as shown in Figure 57.[82] Compound **154** was, however, found to have the best *in vitro* profile having potency of 0.5 x 10^{-6} M and HCR [hypoxic cytotoxicity ratio] of 8.

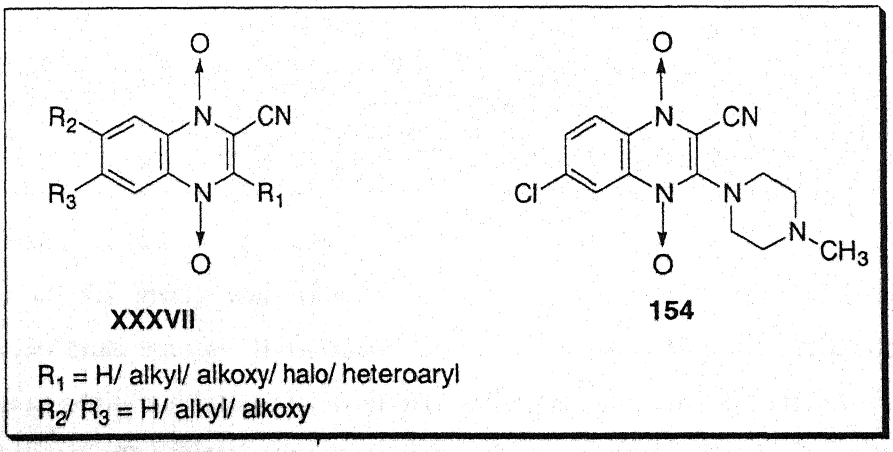


Figure 57: Quinoline prototype **XXXVII** and derivative (compound **154**)

Suh *et al.* synthesized a number of quinoline-5,8-diones of prototypes **XXXVIII** and **XXXIX** and screened for their cytotoxic activity as shown in Figure 58.[83] The cytotoxic activities of quinoline-5,8-diones have been tested in SRB (Sulfo Rhodamine B) assay against the cancer cell lines such as A-549 (lung), SK-MEL-2 (melanoma), SK-OV-3 (ovarian), XF-498 (brain) and HCT-15 (colon). It was found that compound **155** exhibited highest cytotoxicity (IC_{50} value ranges between 0.05-0.08 μg/mL) in comparison to cisplatin but lesser than doxorubicin.[83]

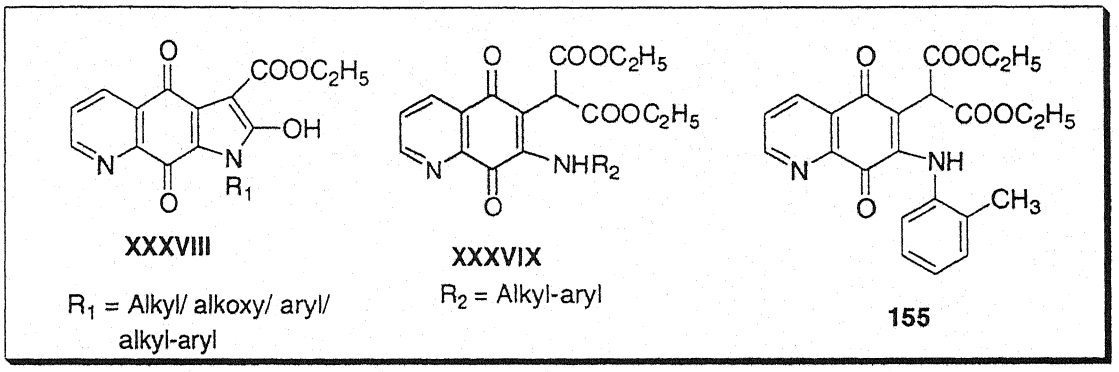


Figure 58: Quinoline prototypes **XXXVIII, XXXXIX** and derivative (compound **155**)

Wissner *et. al.* carried out screening of directed compound library in a yeast based assay.[84] The library consisted of 6,7-dimethoxy-3-quinolinecarbonitriles with various anilino groups at C-4 position. The most effective compound found in restoring cell proliferation in the assay was, compound **156**. The results of the assay led to investigate additional analogues **XXXX** of compound **156** as Src kinase inhibitors as shown in Figure 59.

SAR studies reveal the fact that the anilino group at C-4, the cyano group at C-3 and the alkoxy groups at C-6 and C-7 in the quinoline ring are essential for optimal activity. Increasing the size of the C-2' substituent of the aniline from chloro to bromo to iodo resulted in a corresponding increase in Src inhibition. Furthermore, replacement of the 7-methoxy group with various 3-heteroalkylaminopropoxy groups provided increased inhibition of both Src enzymatic and cellular activity. The replacement of NH group with either O or S or $NHCH_2$ or NHMe or CONH, exhibited a low order activity. Basic amine functionality (water solubilizing group) at C-7 leads to an additional interaction with Src, while the same substitution at C-6 was found detrimental. The most active compound **157** was found to have IC_{50} value of 3.8 nM for the inhibition of Src kinase activity. Compound **157** exhibits

submicromolar activity in inhibiting both the growth of Src transformed rat fibroblasts in suspension and the phosphorylation of Src substrate proteins.

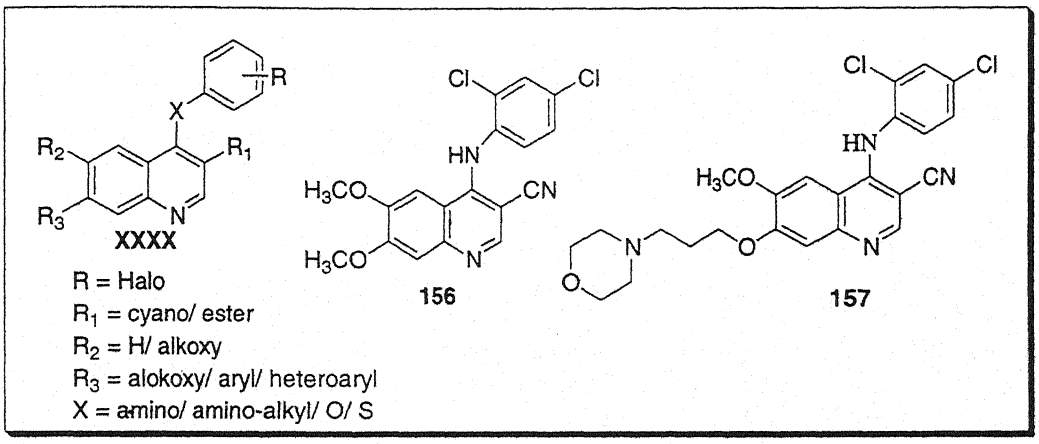


Figure 59: Quinoline prototype **XXXX** and derivatives (compounds **156** and **157**)

Zhang *et. al.* synthesized a series of 4-anilino-3-cyanobenzo[*g*]quinolines of prototype **XXXXI** as potent kinase inhibitors and were compared with their bicyclic analogues of prototype **XXXXII** as shown in Figure 60. It was found that the differential selectivity for these kinases depends on the nature of anilino group at C-4 and dialkoxy substituents at C-6 and C-7 in prototype **XXXXI**. The alkoxy substituents at C-5 and C-8 in prototype **XXXXII** led to decreased activity. A phenyl was fused at C-6 and C-7 of the 3-cyanoquinoline core in prototype **XXXXII** and resulted prototype **XXXXI**.[85]

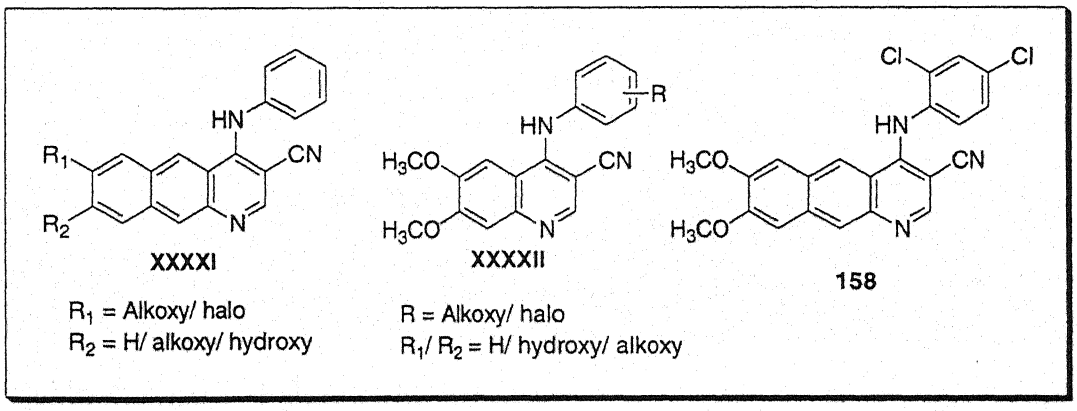


Figure 60: Quinoline prototypes **XXXXI**, **XXXXII** and derivative (compound **158**)

This series of derivatives were found less potent as EGFR inhibitor but was equally potent as MAPK inhibitors and increased potency as Src kinase inhibitors as

compared to their bicyclic analogues against A431 (epidermoid) and SW620 (colon) human carcinoma cell lines. However, compound **158** was found to be the most potent Src kinase inhibitor as IC_{50} value was 0.018 μM in enzymatic assay.

N. Zhang *et. al.* prepared a series of 4-anilino-7-thienyl-3-quinolinecarbonitrile derivatives of prototype **XXXXIII** as Src kinase family inhibitors as shown in igure 61. It has been reported that compound **159** was an ATP competitive inhibitor of Src kinase activity. Modifying the substituents in compound **159** led to compound **160**, which showed potent inhibitor of Src cellular activity. Compound **161** was observed as Src inhibitor and which upon addition of water solubilizing group to the thiophene resulted compounds of prototype **XXXXIII**.[86]

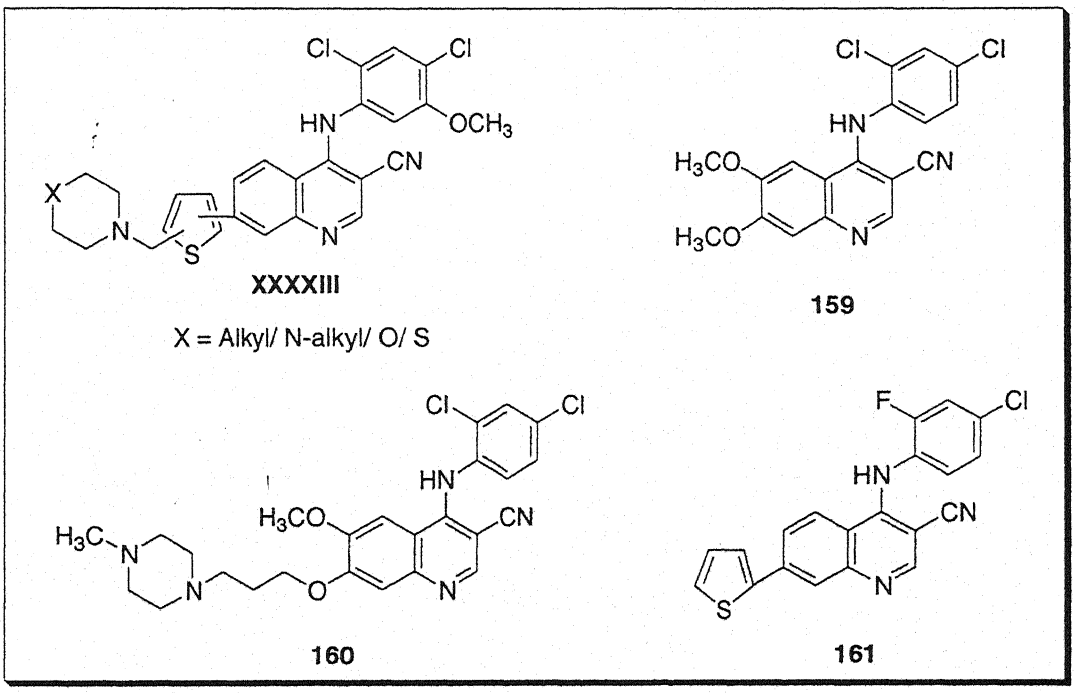


Figure 61: Quinoline prototype **XXXXIII** and derivatives (compounds **159-161**)

In prototype **XXXXIII**, when X = O, wherein the thiophene is substituted with aromatic and morpholine ring in 2',5'- or 2',4'- or 3',5'- fashion showed Src inhibitory activity as IC_{50} value were found to be 2.5 nM , 5.7 nM and 2.7 nM, respectively. In prototype **XXXXIII**, when X = *N*-methyl, wherein the thiophene is substituted with aromatic and *N*-methylpiperazine in 2',5'- or 3',5'- fashion showed 3 fold lower activity than those of the corresponding morpholine analogues. However, these compounds showed to be more potent inhibitor of Src dependent cell

proliferation as IC_{50} value of these compounds were found to be 69 nM and 64 nM, respectively. The SAR studies co-relate that the linear analogues are more potent than their corresponding angular analogues.

Berger *et. al.* synthesized a series of 4-anilino-7-phenyl-3-quinoline carbonitriles of protoype **XXXXIV** as shown in Figure 62, where the phenyl group at C-7 acted as spacer molecule in the similar fashion as 7-thienyl derivatives as reported earlier.[88] Various derivatives were synthesized with water solubilizing substituents attached via different chain lengths (n = 1-2) at either o- , m- or p-positions of phenyl group at C-7. SAR studies reveal the fact that the most active compounds were those derivatives, which possess p- substituted phenyl substituent. None of the compounds with n = 2, had submicromolar activity in the Src dependent cell proliferation assay.

Compounds possessing methoxy group at C-6 position were found less active. It was found that the activity of compound **162** was comparable to compound **163** as IC_{50} values were found to be 71 nM and 100 nM, respectively, against Src-transformed fibroblast cells.

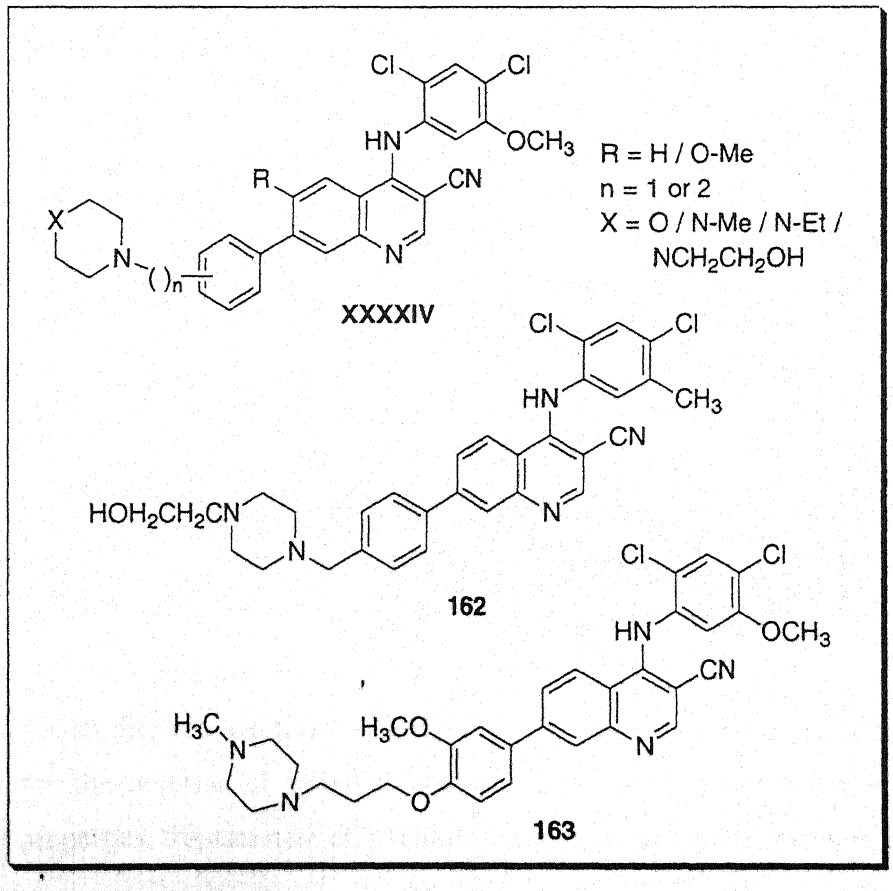


Figure 62: Quinoline prototype **XXXXIV** and derivatives (compounds **162-163**)

Wissner *et. al.* synthesized a series of 6,7-disubstituted-4-anilinoquinoline-3-carbonitrile derivatives of prototype **XXXXV** that functions as irreversible inhibitors of EGFR and HER-2 kinases as shown in Figure 63.[89] Compounds having butynamide, crotonamide, methacrylamide at C-6, were found to be water soluble with improved biological activity. Various modifications at C-4, C-6 and C-7 were carried out and derivatives thus obtained, were tested for their activity against several cancer cell lines. Mostly, three human carcinoma cell lines were used for the evaluation of cytotoxicity such as A431, SKBR3 and SW620. Compound **164** [EKB-596] was emerged as a most potent agent, toxicologically and pharmacokinetically. The IC_{50} value of compound **164** was found to be 0.083 μM against A431 human tumor. It is now selected for Phase I clinical trials for the treatment of EGFR dependent cancers.

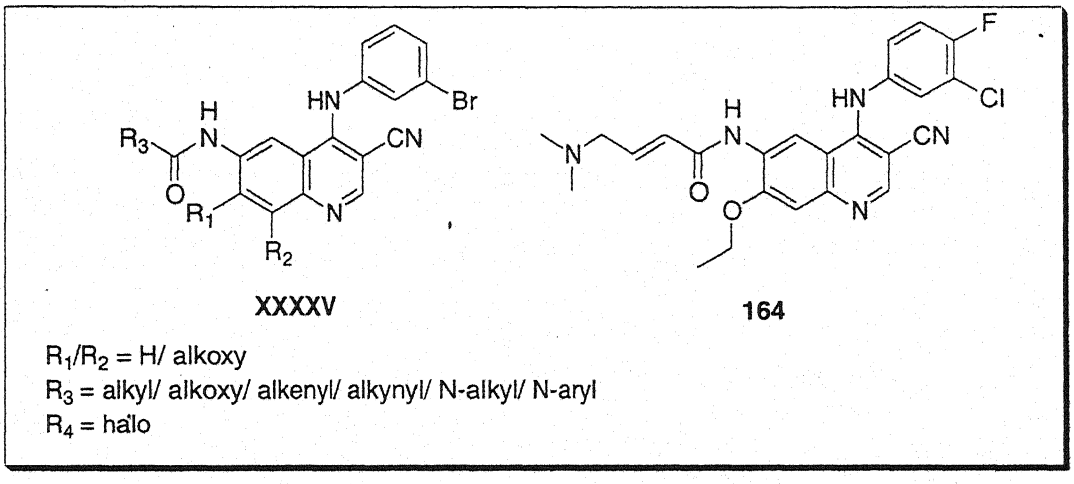


Figure 63: Quinoline prototype **XXXXV** and derivative (compound **164**)

Boschelli *et. al.* synthesized a series of (3'-substituted-4'-(1"-heterosulfonyl))anilino-6,7-dialkoxy-3-quinolinecarbonitrile derivatives of prototype XXXXVI and tested for MEK1 kinase inhibition against LoVo cells as shown in Figure 64.[90] All the compounds were tested in high throughput Raf/ MEK1/ MAPK ELISA assay. These compounds inhibited MEK1 as well as MAPK phosphorylation, thereby acting as a dual inhibitor of the Ras-MAPK signaling cascade.

SAR studies resulted that C-7 position of 4-anilino-3-quinolinecarbonitriles is optimal for the addition of water solubilizing substituents in resulting improved physical properties. Replacement of 3'-chloro substituent on aniline ring with either H

or CH_3 resulted in a modest decrease in activity, while 3'-Br derivative was found equipotent.

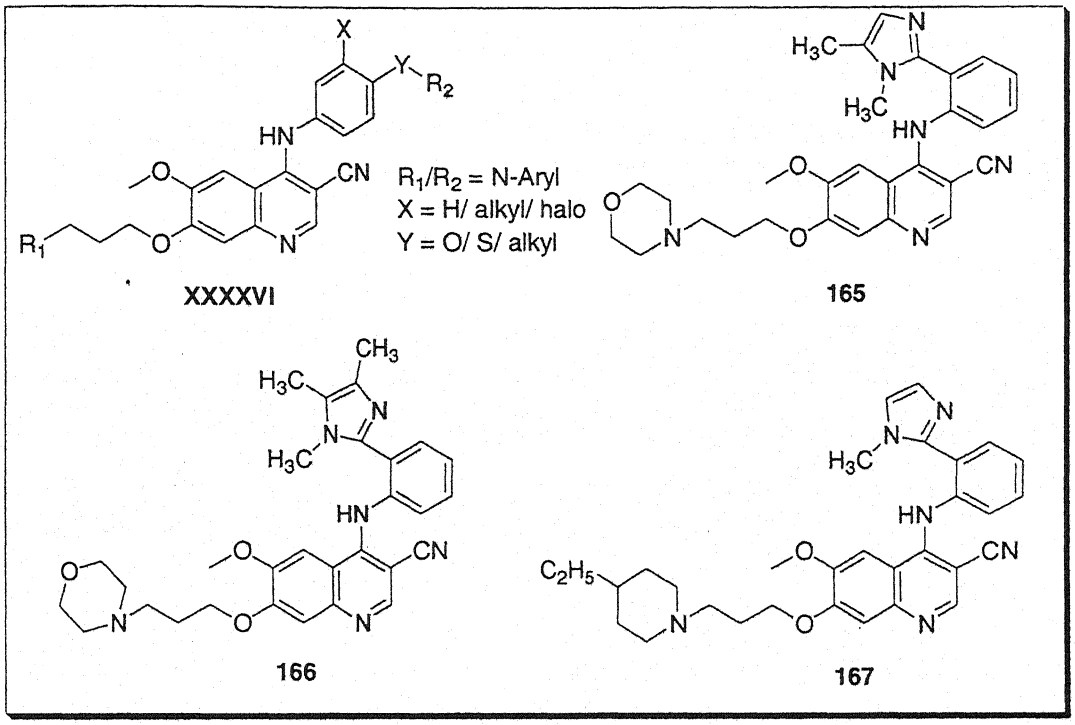


Figure 64: Quinoline prototype **XXXXVI** and derivatives (compounds **165-167**)

However, compounds **165** and **166** were found highly active in enzyme assay (Raf / MEK1) with IC_{50} value of 1 nM and 2 nM, respectively, but modest activity was observed against LoVo cells with IC_{50} value of 325 nM and 342 nM, respectively. Compound **167** showed *in vivo* activity against LoVo cells as IC_{50} value was found to be 7 nM while in case of Raf / MEK1, IC_{50} value was 3 nM.

Boschelli *et. al.* synthesized and evaluated several 7-alkoxy-4-anilino-3-quinolinecarbonitrile derivatives of prototype **XXXXVII** for Src kinase inhibitory activity as shown in Figure 65.[91]

This study was based on the earlier reports, that 3-quinolinecarbonitriles substituted at C-4 with either 2,4-dichloroanilino or 3,4,5-trimethoxyanilino or 2,4-dichloro-5-methoxyanilino groups inhibited Src enzymatic and cell activity. It was found that some compounds had shown fairly comparable activity in the Src cell assay and were chosen for protein kinase study. However, compound **168** exhibited superior plasma level and had elicited Src IC_{50} value of 2 nM and Src cells IC_{50} value of 220 nM against Src transformed fibroblast xenograft.

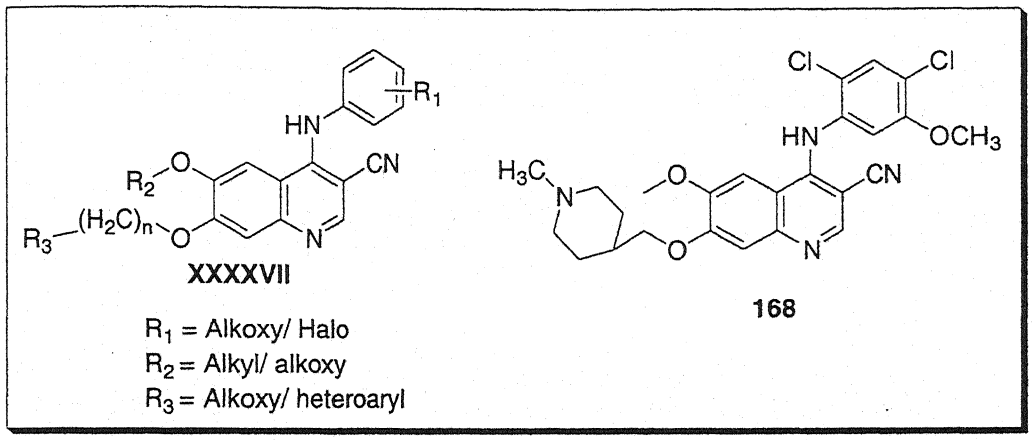


Figure 65: Quinoline prototype **XXXXVII** and derivative (compound **168**)

Y. L. Zhao *et. al.* synthesized a number of 3'- or 4'-substituted-4-anilino-2-phenyl quinoline derivatives of prototype **XXXXVIII** and evaluated for the cell growth inhibition as shown in Figure 66.[92]

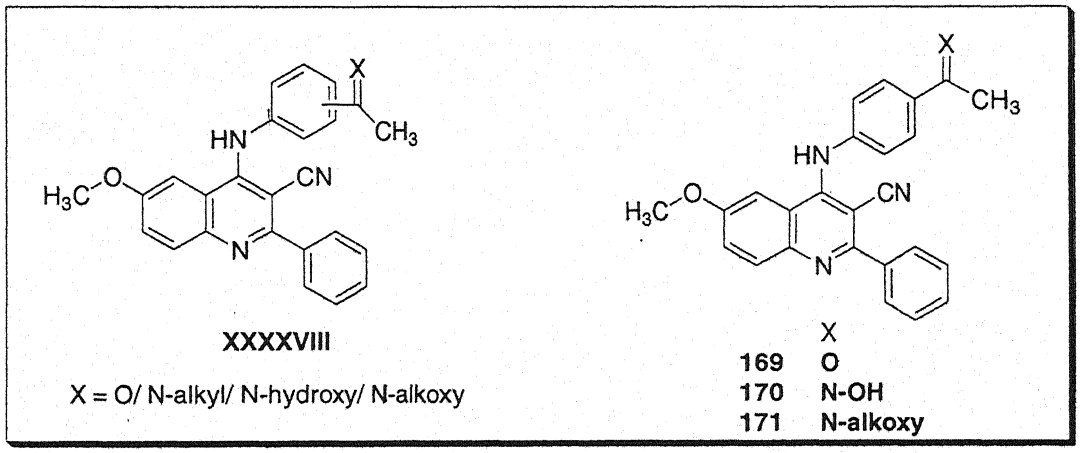


Figure 66: Quinoline prototype **XXXXVIII** and derivatives (compounds **169-171**)

These derivatives were evaluated for their cytotoxicity against 60 cancer cell lines. The preliminary cytotoxic assay indicated that 4'-substituted-4-anilino derivatives are more effective than their respective 3'-substituted-4-anilino derivatives.

Compounds **169, 170** and **171** showed significant cytotoxicity against all 60 cancer cell lines with mean GI_{50} value of 3.89 μM, 3.02 μM and 3.89 μM, respectively. The comparable cytotoxicity of oxime (**170**), methyloxime (**171**) and the ketone precursor (**169**) implied that a hydrogen bonding accepting group at C-4'

position of 4-anilino moiety is crucial for cytotoxicity. Compound **169** was especially active against growth of certain solid cancer cells such as NCI-H226, MDA-MB-231/ATCC, SF-295 with GI_{50} value of 0.94 μM, 0.04 μM and 0.01 μM, respectively.[92]

Hazeldine *et. al.* synthesized a number of derivatives of prototype **XXXXIX** and screened for their activity against transplanted tumors like Colon38 and Panc03 in mice as shown in Figure 67.[93] SAR studies reveal the fact that, intact quinoxaline or quinoline rings are essential for the activity. Modifications on the parent structure with either 1,8-naphthyridine or phenanthridine or pyrrolo[1,2-*a*] or imidazo[1,2-*a*] or [1,5-*a*] led the parent structure deprive of the activity. Presence of a halo group at C-7 was essential for eliciting the cytotoxicity.

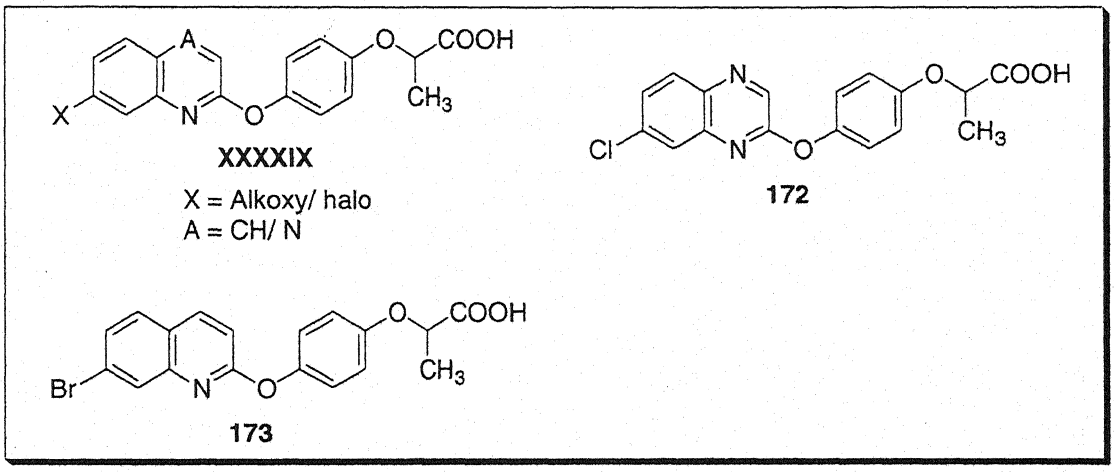


Figure 67: Quinoline prototype **XXXXIX** and derivatives (compounds **172** and **173**)

When A is substituted by nitrogen, the relative anti-tumor activity of 7-halo derivatives were found in the order of Cl ≈ Br ≈ F > I. While if A is replaced by CH, the relative anti-tumor activity of 7-halo derivatives were found in the order of Br > Cl > F ≈ I. Upon introduction of CH_3, CF_3, OCH_3, COOH or C_6H_5 substituents at C-4 in quinoline ring led to a group of weakly active compounds. Studies were also carried out for regioisomeric structures. Compounds **172** and **173** were identified as the most potent derivatives. The 3-, 5-, 6- and 8- regioisomers of **173** [XK-469] were essentially inactive, while 4-regioisomer of **173** [SH-80] was less active than **173**.

Kubo *et. al.* synthesized a series of N-phenyl-N'-{4-(4-quinolyloxy)phenyl}urea derivatives of prototype **XXXXX** as shown in Figure 68. Through synthetic modifications of lead compound **174** and SAR studies, compound **175** was found to be most potent VEGFR-2 phosphorylation inhibitor as the IC_{50} value was 0.9 nM.[94]

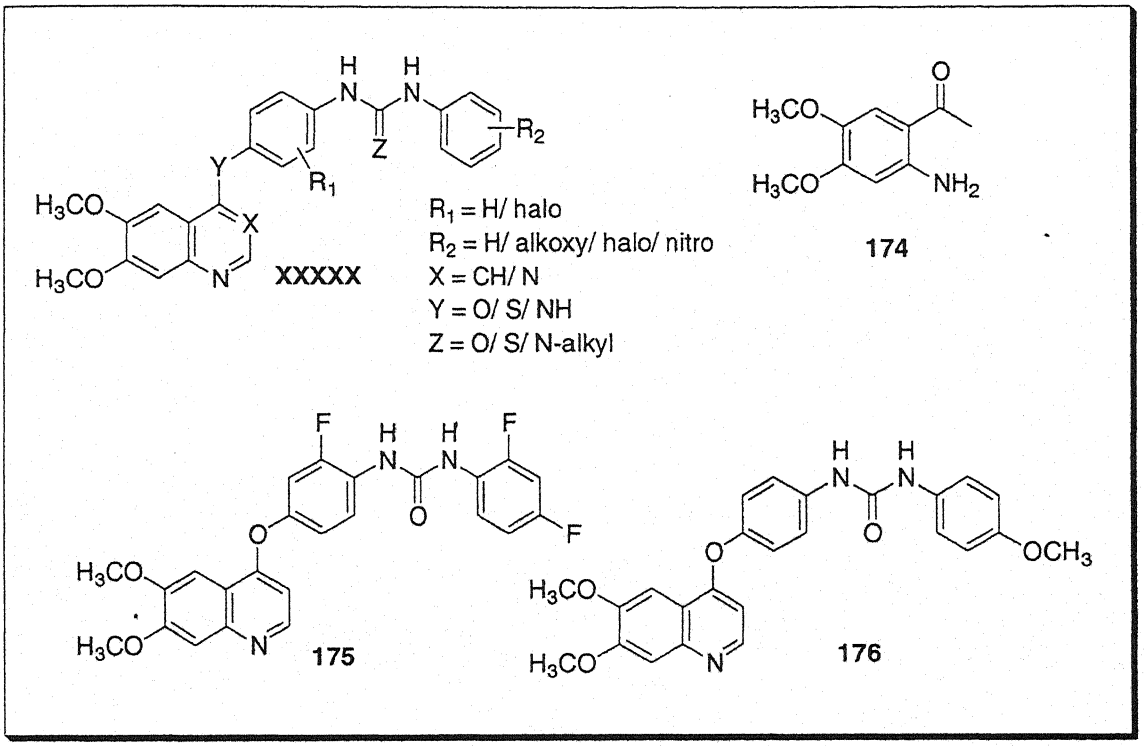


Figure 68: Quinoline prototype **XXXXX** and derivatives (compounds **174-176**)

Compound **175** also inhibited PDGFR family members such as PDGFR-α and c-Kit at 67 nM and 40 nM, respectively against five human tumor xenograft such as GL07, St-4, LC-6, DLD-1 and A375. It also showed complete tumor growth inhibition with the LC-6 xenograft at an oral administration for 14 days at 5 mg/Kg body wt. IC_{50} value of compound **176** against VEGFR-2 and PDGFR-α was found to be 1.1 nM and 2.0 nM, respectively. When X = CH, Y = S or NH and Z = O, activity decreased. When X = CH, Y = O and Z = S, activity decreased for VEGFR-2. Compounds having X = CH, Y = O and Z = NCH, showed loss of activity for PDGFR-α.

Tsou *et. al.* synthesized a series of new 6,7-disubstituted–4-(arylamino) quinoline-3-carbonitrile derivatives of prototype **XXXXXI** that function as irreversible inhibitors of HEGFR-2 (HER-2) and EGFR kinases as shown in Figure 69.[95] SAR studies reveal the fact that the large lipophilic group substituted at p-

position of arylamino ring showed' enhanced potency for inhibiting HER-2 kinase. This study resulted in compound **177** [HKI-272], which was most potent against HER-2 and equally potent for EGFR. Compound **177** showed IC_{50} value for HER-2 and EGFR as 0.059 μM and 0.092 μM, respectively. The most potent derivative for EGFR was found to be compound **178** [EKB569] with IC_{50} value of 0.08 μM. *In vivo* studies in BT474 and SUM190 tumor cells. Compound **177** showed better activity than **178** at a dose of 10 mg/Kg. On the basis of extensive pharmacokinetics and toxicological studies, compound **177** showed best activity and now is in Phase I clinical trial.

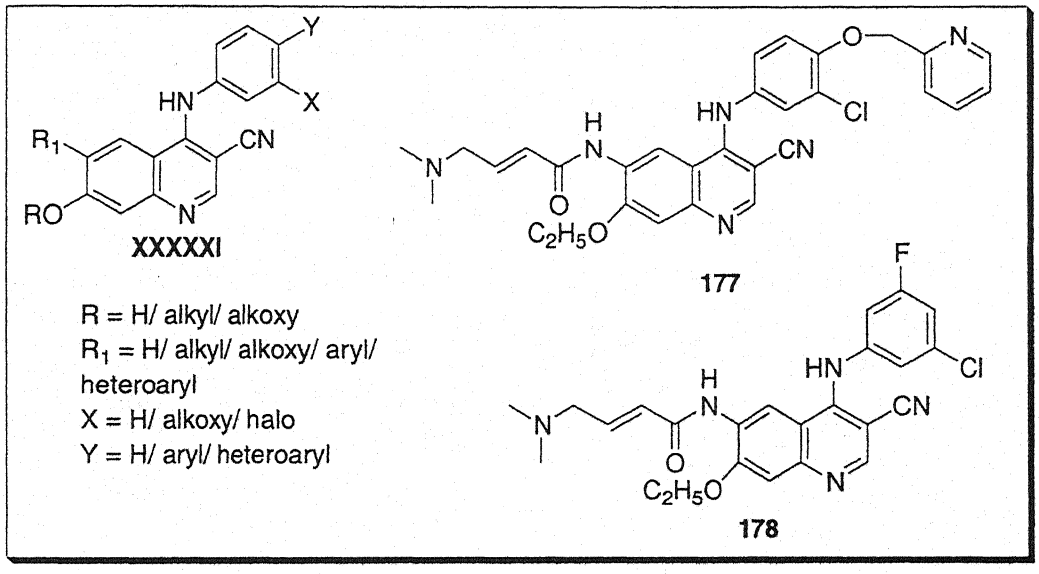


Figure 69: Quinoline prototype **XXXXXI** and derivatives (compounds **177** and **178**)

1.6 1,8-Naphthyridine Derivatives as Anticancer

Tomita *et. al.* synthesized a number of derivatives of 7-substituted-1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acid of prototype **XXXXXII** and screened for their anti-tumor activity as shown in Figure 70.[96]

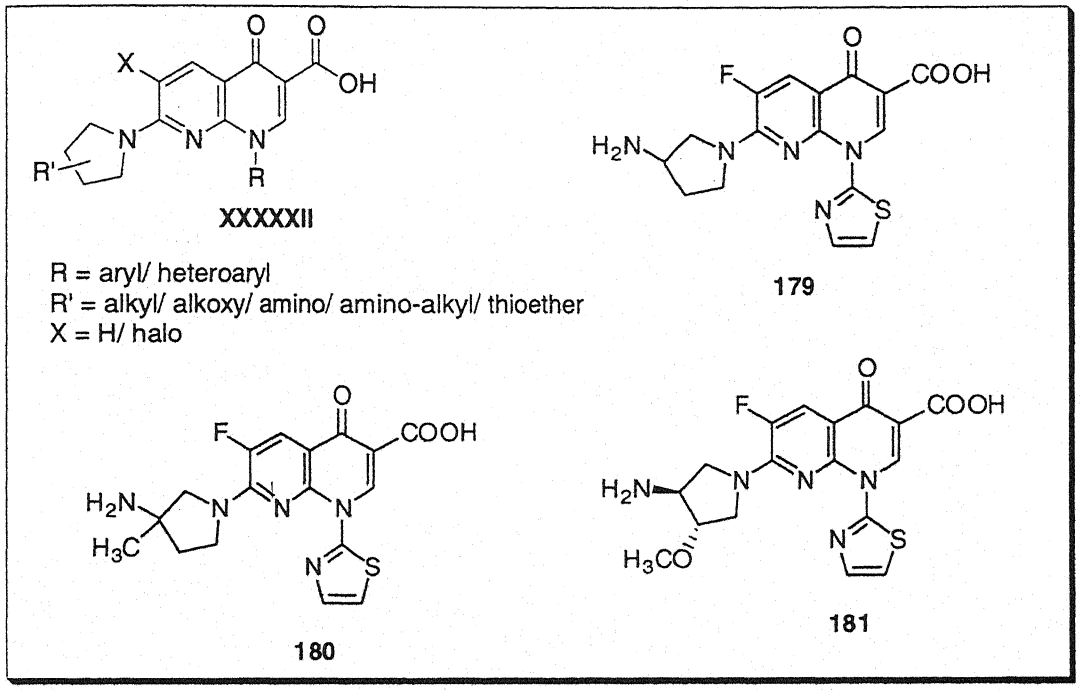


Figure 70: 1,8-Naphthyridine prototype **XXXXXII** and derivatives (compounds **179-181**)

SAR studies for these derivatives were carried out by changing the substituents at C-6, C-7, N-1 and also at the core ring structure. The studies reveal the fact that the 2-thiazolyl at N-1 shows to be the best substituent for anti-tumor activity. At C-7 position, aminopyrrolidine functionality was found to be more effective than any other amine or thioether derivatives. However, compounds **179**, **180** and **181** were found to be effective in *in vitro* and *in vivo* anti-tumor assays and their anticancer activity was compared with etoposide and doxorubicin. Compound **179** showed IC_{50} value of 0.021 μg/mL against murine P388 leukemia, while compounds **180** and **181** showed IC_{50} value of 0.038 μg/mL and 0.026 μg/mL against the same cell line, respectively. Compounds **179** and **181** both displayed significant cytotoxicity but slightly less than etoposide against various human tumor cell lines such as G-361 (melanoma), AZ-521 (stomach), HT-29 (colon), A-427 (lung) and SK-OV-3 (ovary).

Tomita *et. al*, has further synthesized and studied the SAR of 3-substituted-1,4-dihydro-4(oxo)-1-(2-thiazolyl)-1,8-naphthyridines of prototype **XXXXXIII** as shown in Figure 71.

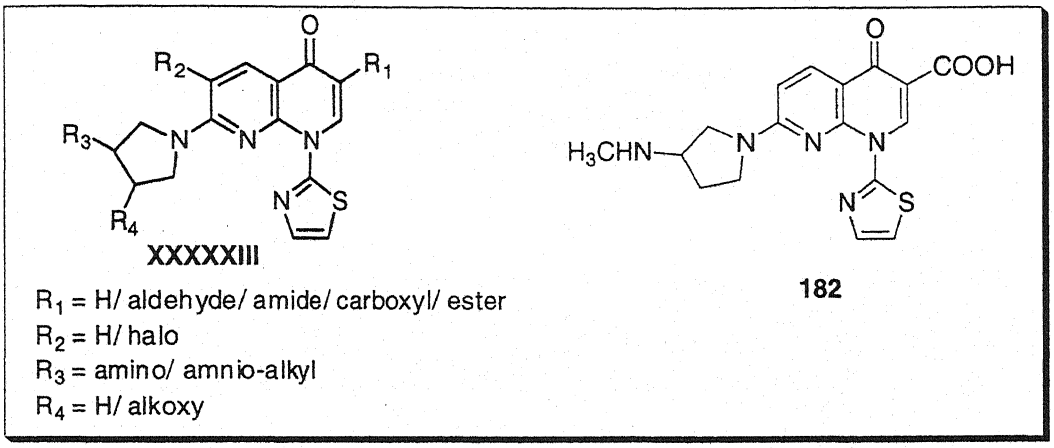


Figure 71: 1,8-Naphthyridine prototype **XXXXXIII** and derivative (compound **182**)

These novel derivatives showed anti-tumor activity against murine P388 leukemia, human tumor cell lines as well as *in vivo* for mouse leukemia. Compound **182** was the most potent having IC_{50} value of 0.0104 μg/mL against murine P388 leukemia. SAR studies indicated that 3-formyl derivative showed activity equal to that of hydroxy carbonyl derivative.

Hence, it was argued that carboxylic acid group at C-3 position in 1,8-naphthyridine is not essential for eliciting the cytotoxicity. Modifications at C-6 and C-7 positions showed good anti-tumor activity against murine leukemia.[97]

Tomita *et. al.* has further synthesized and studied a series of 7-substituted-6-fluoro-1,4-dihydro-4(oxo)-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acids of prototype **XXXXXIV** and evaluated for their anticancer activity as shown in Figure 72.

SAR studies was carried out by changing the substituents at C-5, C-6 and C-7 positions and evaluated against several murine and human tumor cell lines. It was found that C-6-desfluoro-1,8-naphthyridine analogues had shown better cytotoxicity against murine P388 leukemia, which was found to be twice that of 6-fluoro analogue. No effect was observed upon substitution of amino group at C-5 while both chloro and trifluoromethyl group at position-5 decreased the cytotoxic activity by five to ten folds. Aminopyrrolidine substituent at C-7 exhibited more potent cytotoxic activity

than other amines or carbon derivatives. Finally, compound **183** was identified as most potent cytotoxic agent in both *in vitro* and *in vivo* assays. Compound **183** also showed higher water solubility. Further, it was observed that (S,S)-isomer of AG7352 showed 2.8-fold higher cytotoxicity than (R,R)-isomer against human tumor cell lines. IC_{50} value of (S,S)-isomer and (R,R)-isomer of compound **183** was found to be 0.0075 μg/mL and 0.021 μg/mL, respectively. Compound **183** was later licensed to Sunesis for further development and coded as SNS-595. The compound SNS-595 is currently in Phase II clinical trials.[98]

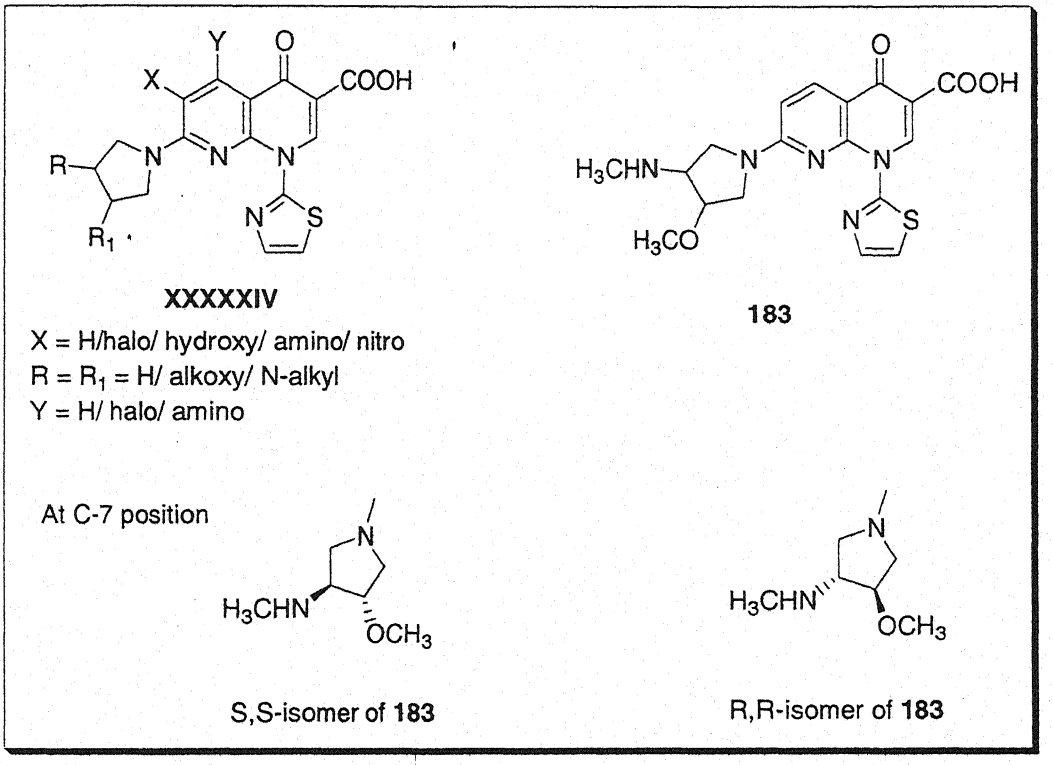


Figure 72: 1,8-Naphthyridine prototype **XXXXXIV** and derivative (compound **183**)

Agarwal *et al.* has synthesized various naphthyridine derivatives of prototype **XXXXXV** by carrying out the substitutions of different amino acid at C-3 position in 1,8-naphthyridine molecule as shown in Figure 73.[99]

These 1,8-naphthyridine-3-carboxamide derivatives showed both anticancer and anti-inflammatory activities. Among these derivatives compounds **184**, **185** and **186** have shown potent anticancer activity. Compound **184** has shown IC_{50} value of 1.37 μM in HBL100 (breast) cancer cell line. Compounds **185** and **186** have shown

IC_{50} value of 3.7 and 3.0 μM on KB (oral) and SW-620 (oral) cancer cell lines, respectively.

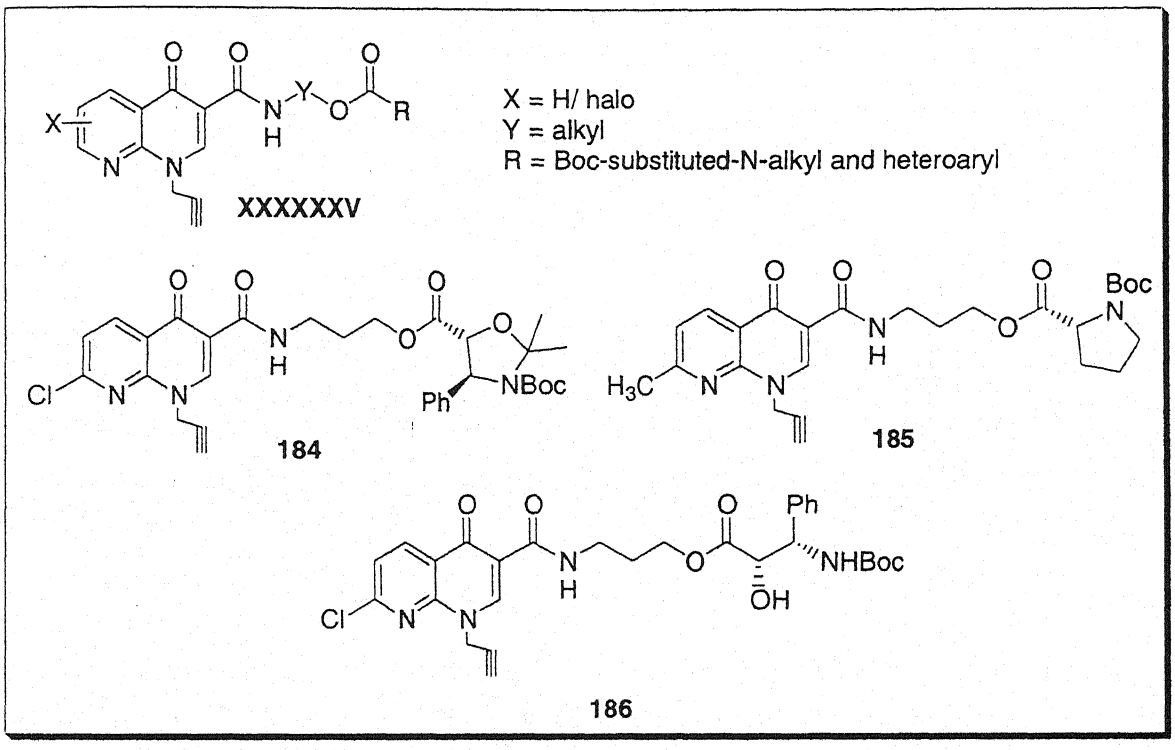


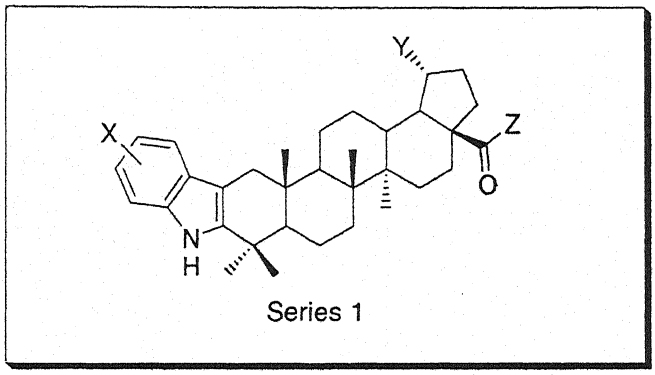
Figure 73: 1,8-Naphthyridine prototype **XXXXXV** and derivatives (compound **184-186**)

CHAPTER-2

OBJECTIVES

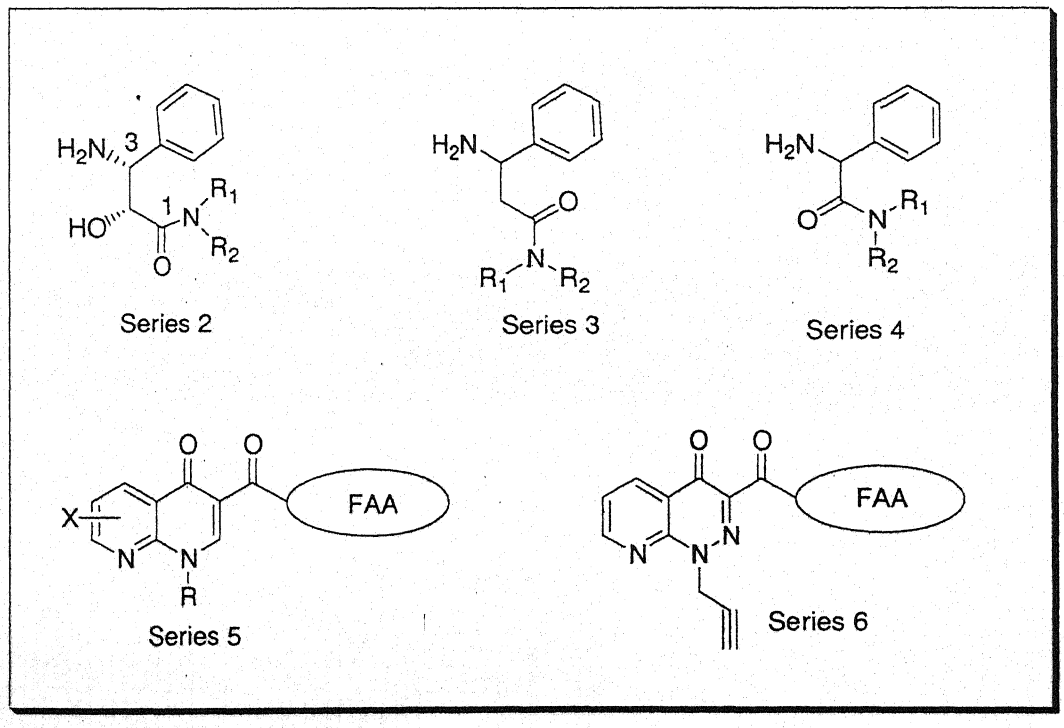
2. OBJECTIVES

2.1. Synthesis of novel betulinic acid derivatives: To synthesize novel betulinic acid derivatives of Series 1, which are expected to exhibit better cytotoxicity and improved pharmacokinetics.



Series 1

2.2. Synthesis of functionalized amino acids and novel 1,8-naphthyridine-3-carboxamide derivatives: To synthesize various novel 1,8 naphthyridine-3-carboxamide derivatives of Series 5 and 6, with expected improved cytotoxicity and immunomodulatory activity, by using different functionalized amino acid (Series 2-4) at position C-3.



2.3. Cytotoxicity and anti-inflammatory activity: To evaluate the *in vitro* cytotoxicity and anti-inflammatory activity of the synthesized compounds.

CHAPTER-3

RESEARCH ENVISAGE

3. RESEARCH ENVISAGE

3.1 Betulinic acid derivatives:

A series of betulinic acid derivatives with different cytotoxic activities have been synthesized in past by different groups. To further improve the activity of betulinic acid various modifications were carried out at different positions. However, due to various reasons they are not particularly clinically good candidates, as well as do not have the best of pharmacokinetic properties. By inducing heterocyclic indole group at C-2 and C-3 positions of betulinic acid, the effect of variation in hydrogen bonding potential, pKa, lipophilicity and selectivity was observed. Further changes are proposed by making the modifications at C-20 unsaturated bond and C-28 carboxylic functional group.

3.2 Functionalized amino acids and 1,8-Naphthyridine-3-carboxamide derivatives:

Both of the well known anticancer molecules Paclitaxel and Docetaxel are having amino acids side chain, based on which we proposed to synthesize different amino acids and to test them separately for their anticancer activity to find out potent molecules. These molecules can be further used as new anticancer pharmacophore or as side chains. 1,8-Naphtyridine derivatives were found to display moderate cytotoxic activity against murine P388 leukemia, when changes were carried out at N-1 and C-7 position. We have proposed to carry out further changes at C-3 position and to synthesize various 1,8-naphthyridine-3-carboxamide derivatives, and to test them against different cancer cell lines. These compounds may show promising anticancer activities and selected will be further tested for their potential anti-inflammatory activity based on the molecular link between cancer and inflammation.

CHAPTER-4

RESULTS AND DISCUSSION

4. RESULTS & DISCUSSION

4.1 Synthesis of novel betulinic acid derivatives:

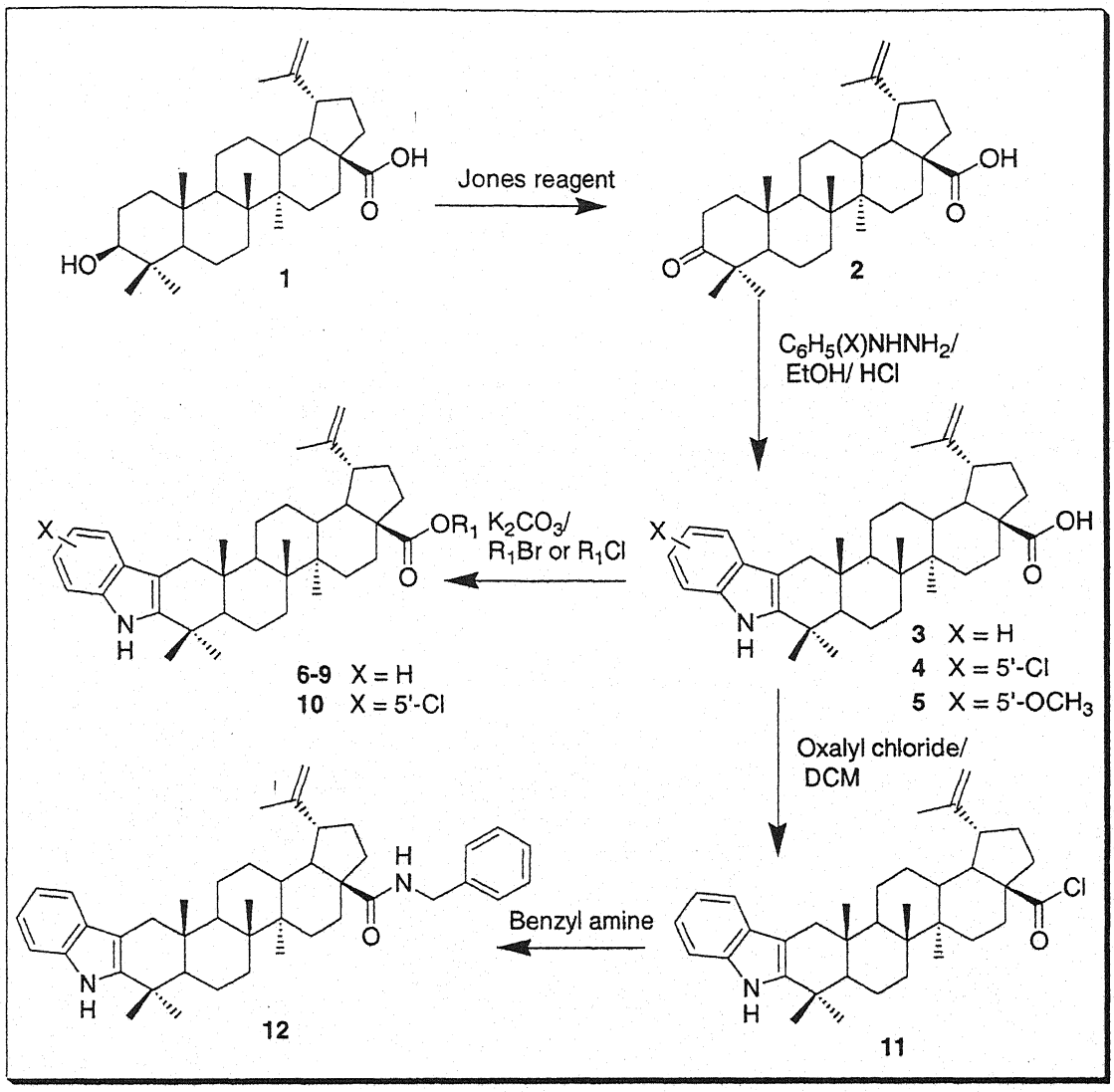
A Series of betulinic acid derivatives with different cytotoxic activities have been synthesized in past by different groups. To further improve the activity of betulinic acid various modifications were carried out at different positions. However, due to various reasons they are not particularly clinically good candidates, as well as do not have the best of pharmacokinetic properties. By inducing heterocyclic indole group at C-2 and C-3 positions of betulinic acid, the effect of variation in hydrogen bonding potential, pKa, lipophilicity and selectivity was observed. Further changes were also carried out by making the modifications at C-20 unsaturated bond and C-28 carboxylic functional group.

4.1.1 Synthesis and Characterization of betulinic acid derivatives

Series 1 (Scheme 1):

Betulinic acid was oxidized in presence of Jones reagent to afford betulonic acid (**2**). Compound **2**, ^{1}H NMR spectra showed a disappearance of multiplet at δ 3.14 ppm for H-3 proton. Further, there was no effect on the methylene protons peaks, which remained as such at δ 4.61 ppm and δ 4.74 ppm. The EIMS (-) spectral data has showed molecular ion peak m/z at 453. Compound **2** undergo cyclization with unsubstituted/ substituted phenylhydrazine under acidic condition in presence of alcoholic solvent to furnish indolo derivative (**3-5**). Compounds **3-5** have showed characteristic peaks for the aromatic proton in NMR, in region of δ 7-8 ppm. Compound **3** and **4** were converted to acyl derivatives (**6-10**) by reacting with alkyl halide in basic condition. To obtain the amide derivative (**12**) compound **3** was converted to acyl chloride (**11**) in presence of oxalyl chloride. Compound **11** was treated with benzyl amine to afford the amide derivative (**12**).

Scheme 1: Synthesis of betulinic acid derivatives



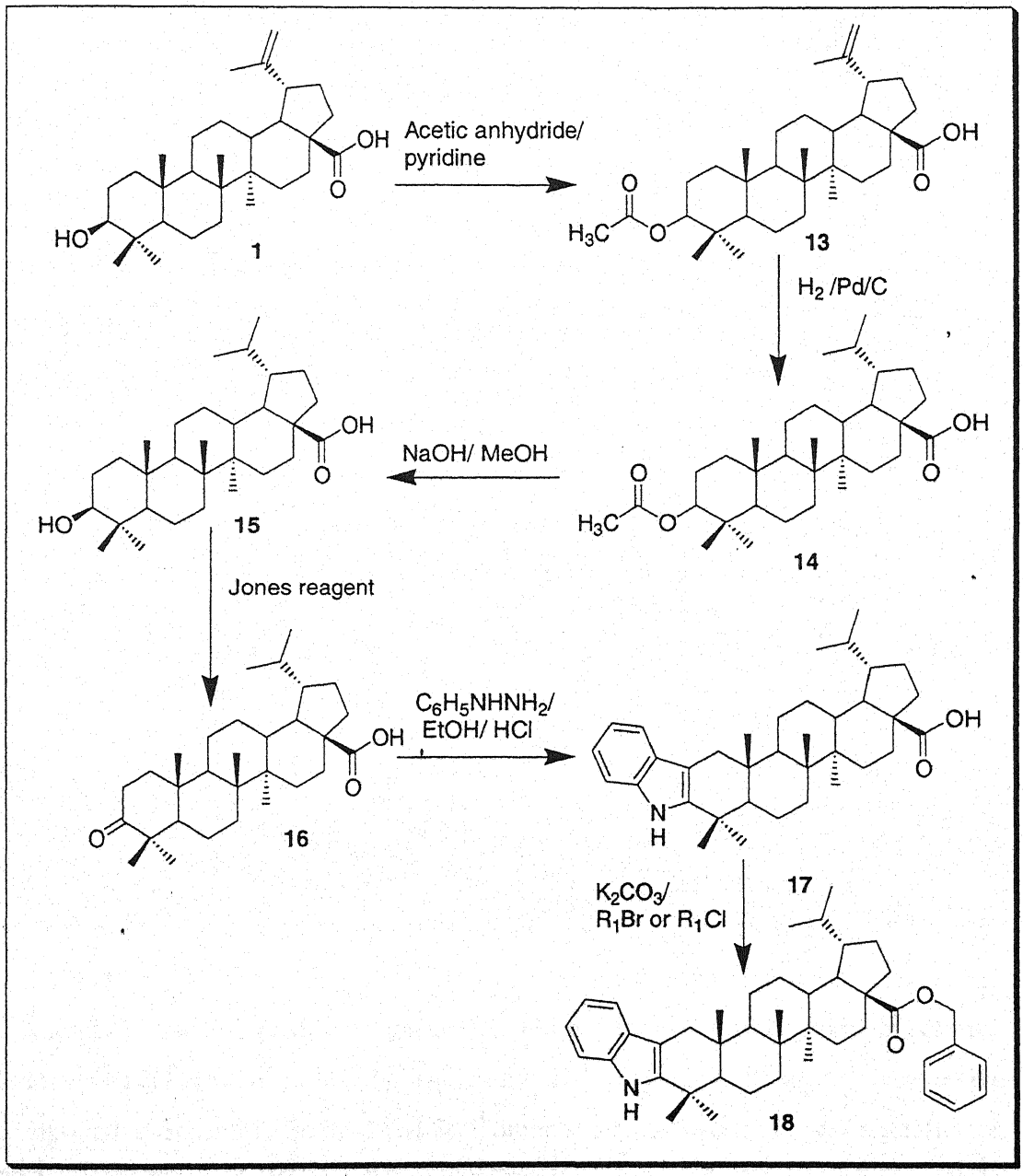
4.1.2 Synthesis and Characterization of betulinic acid derivatives

Series 1 (Scheme 2):

Betulinic acid was acetylated with acetic anhydride in presence of pyridine to afford 3-*O*-acetyl betulinic acid (**13**). Compound **13**, ^{1}H NMR spectra showed a singlet at δ 2.04 ppm for $-COCH_3$ protons. The EIMS (-) spectral data has showed molecular ion peak m/z at 497.Compound **13**, on hydrogenation with Pd/C in hydrogen gas afforded 3-*O*-acetyl 20,29-dihydro betulinic acid (**14**), its ^{1}H NMR spectra showed a disappearance of singlets at δ 4.61 ppm and at δ 4.73 ppm for methylene protons. The 20,29-dihydrobetulinic acid (**15**) was synthesized by deacetylation of 3-*O*-acetyl 20,29-dihydrobetulinic acid (**13**) with methanolic sodium hydroxide. ^{1}H NMR spectra of compound **15** showed a disappearance of singlet at δ

2.04 ppm for $-COCH_3$ protons as well as appearance of a peak at δ 3.14 ppm for H-3 proton. The EIMS (-) spectral data has showed molecular ion peak m/z at 457.6. Compound **15** was oxidized to compound **16**, using Jones reagent as in Scheme 1. ^1H NMR spectra showed a disappearance of multiplet at δ 3.14 ppm for H-3 proton. The EIMS (-) spectral data has showed molecular ion peak m/z at 455.5. Compound **16** was converted to its indolo derivative (**17**) as similar to Scheme 1 but with absence of the peak for mrthylene protons. Compound **17** was esterified at C-28 position under basic condition to compound **18**.

Scheme 2: Synthesis of betulinic acid derivatives



4.1.3 Biological evaluation of betulinic acid derivatives Series 1 (Scheme 1 and 2):

The structures of the compounds are shown in Table1. These compounds were tested for cytotoxicity against prostate, lungs, laryngeal, pancreas, breast, colon and ovarian cancer, leukemia and lymphoma, human tumor cell lines as shown in Table 2. Briefly, a three day MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) cytotoxicity assay was performed, which is based on the principle of uptake of MTT, a tetrazolium salt, by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically.[101] MTT was dissolved in phosphate buffered saline with a pH of 7.4 to obtain an MTT concentration of 5mg/ml; the resulting mixture was filtered through a 0.22-micron filter to sterilize and remove a small amount of insoluble residue. For each type of tumor cell, 10,000 to 15,000 cells were seeded in a 96-well culture plate and incubated with the individual betulinic acid derivatives in a CO_2 incubator for a total of 72 hours. Control cells not treated with the betulinic acid derivatives were similarly incubated. The assay was terminated by adding 100 μg (20 μl) of MTT to each well, then incubating for additional one hour, and finally adding 50 μl of 10% SDS-0.01N HCl to each well to lyse the cells and dissolve formazan. After incubating for one hour, the plate was read spectrophotometrically at 540 nm and the percentage of cytotoxicity calculated using the following formula:

Cytotoxicity percentage = 100 x [1-(X/R_1)],

where X = (absorbance of treated sample at 540 nm) - (absorbance of blank at 540 nm)

R_1 = absorbance of control sample at 540 nm.

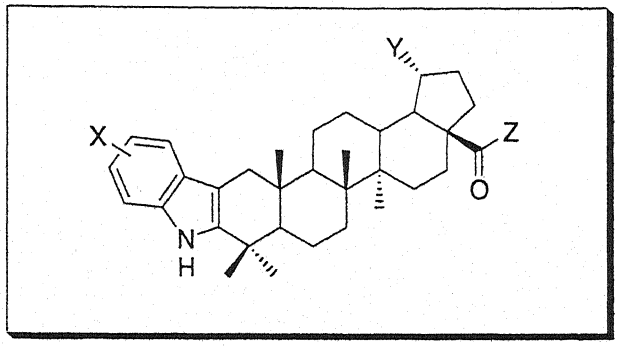
The IC_{50} values of the cytotoxicity defined as the concentration at which 50 % of the cells are killed *in vitro* was calculated for each cell line treated with each of the betulinic acid derivatives.

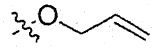
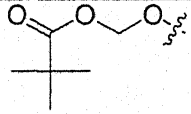
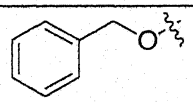
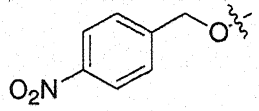
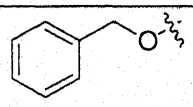
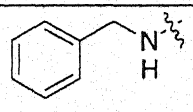
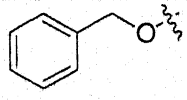
4.1.4 Structure activity relationship (SAR):

These betulinic derivatives have revealed that by inducing heterocyclic group indole at C-2 and C-3 positions of betulinic acid has showed improvement in activity. Indolo ring-substituted carboxylic acid derivative (compound **5**) is the most active compound of Scheme1 (Series 1 and 2). Compound **5** has two times more activity on PA1 cancer line with IC_{50} of 5.8 µg/ml (betulinic acid with IC_{50} of 11.53 µg/ml).

Major enhancements in the cytotoxicity was observed on SW620 and Miapaca cancer cell lines with IC_{50} of 8.4 and 6.4 μg/ml, respectively.

Table 1: Betulinic acid derivatives (Series 1)



S.No.	Compd. No.	X	Y	Z
1	**5**	5'-OCH_3		OH
2	**6**	H		
3	**7**	H		
4	**8**	H		
5	**9**	H		
6	**10**	5'-Cl		
7	**12**	H		
8	**18**	H		

When the C-28 carboxylic group was esterified with allyl group (compound **6**) or methyl pivalate (compound **7**) no enhancement in the activity was observed. Further,

substitution with benzyl (compound **8**) and benzyl substituted with electron withdrawing group (compound **9**) caused the loss in the activity.

Table 2: IC_{50} values of in vitro cytotoxicity of betulinic acid derivatives (Series 1)

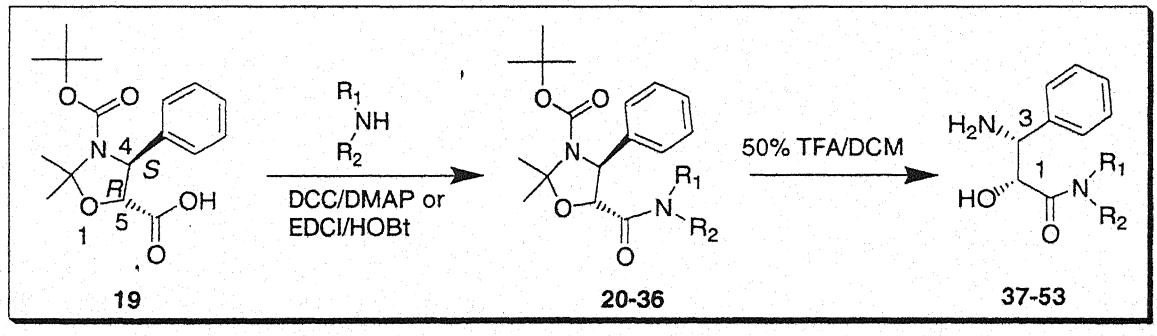
Compound. No.	IC50 (µg/ml) for cell lines							
	NIH3T3	PA1	DU145	SW620	HBL100	Miapaca	A549	K562
Betulinic acid	-	11.53	>20	13.26	5.02	>20	3.008	-
5	7.5 ± 0.48	5.8	5.75	8.4	12.8 ± 0.39	6.4 ± 0.44	8.2 ± 0.14	>20
6	>20	>20	>20	>20	>20	>20	>20	>20
7	>20	>20	>20	>20	>20	>20	>20	>20
8	>20	>20	>20	>20	>20	>20	>20	>20
9	>20	>20	>20	>20	>20	>20	>20	>20
10	>20	>20	>20	>20	>20	>20	>20	>20
11	>20	>20	>20	>20	>20	>20	>20	>20
18	>20	>20	>20	>20	>20	>20	>20	>20

On derivatisation of compound **8** with halo group at C-5' position leads to inactive compound **10**. Ester linkage was further replaced by amide group (compound **12**) to see the effect of this acidic group on cytotoxicity but no enhancement in the activity was observed. Similarly, hydrogenation at C-28 position in compound **8** has caused no variation in the cytotoxicity (compound **18**).

4.2. Synthesis of functionalized amino acids and novel 1,8-naphthyridine-3-carboxamide derivatives:

Tomita *et al.* has synthesized various 1,8-naphthyridine derivatives as antitumor agents by carrying out various modifications at N-1, C-2, C-5, C-6 and C-7 positions.[96,97] In our efforts to find out a potent molecule and to understand SAR in 1,8-naphthyridine derivatives, we have focused on the C-3 position and modified the C-3 carboxylic acid with different functionalized amino (**37-53, 56-63, 66-71**) acids to afford 1,8-naphthyridine-3-carboxamide derivatives along with the conversion of 1,8-naphthyridine ring to pyrido[2,3-*c*]pyridazine ring system. The amide linkage may provide hydrophilic interaction while functionalized amino acids may interact with the receptors and as a consequence, it could trigger physiological response. Herein, we report the synthesis and *in vitro* cytotoxicity of 1,8-naphthyridine derivatives. Few of the molecules synthesized were also investigated for their potential anti-inflammatory activity using an *in vitro* septic shock assay based on murine bone marrow-DCs, as indicated by resultant down regulation of various pro-inflammatory cytokines.

4.2.1 Synthesis and characterization of functionalized amino acid derivatives (Series 2, Scheme 3) (20-53):



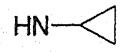
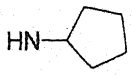
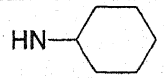
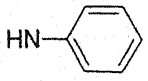
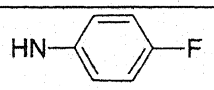
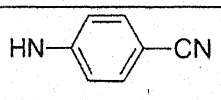
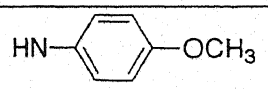
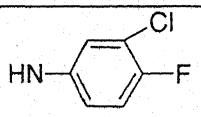
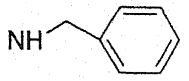
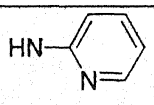
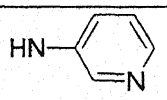
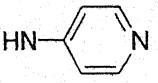
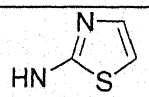
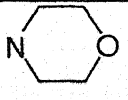
Coupling of *4S,5R*- 1-*N*-(tert-butoxycarbonyl)- 2,2-dimethyl-4-phenyl-5-oxazolidine carboxylic acid (**19**)[100] with appropriate amines has been carried out to afford the respective *N*-substituted-1-*N*-(tert-butoxycarbonyl)-2,2-dimethyl-4-phenyl-5-oxazolidine carboxamide (**20-36**). The coupling reactions were performed by either *N,N'*,-dicyclohexyl carbodiimide (DCC) and dimethylaminopyridine (DMAP) or *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) in DCM or DMF, which were used as solvent. Formation of amide bond was

confirmed by the ^{1}HNMR signals and EIMS study. Coupling constant (*J*) in between the protons at C-4 and C-5 was 5-6 Hz.

The oxazolidine ring of compounds **20-36**, was separately, opened with 50% TFA/DCM to afford corresponding 1-*N*-substituted-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide (**37-53**). The functionalized amino acid derivatives (**20-53**) of Series 4 are listed in Table 3. Coupling constant (*J*) in between the protons at C-4 and C-5 was 2-4 Hz.

Compounds **25-28, 35, 43-45, 47, 50** and **51** were synthesized by the other members of medicinal chemistry division. These molecules were further scaled up and characterized to use them as side chain.

Table-3: Functionalized amino acid derivatives (**20-53**)

Compound No.	NR_1R_2	Compound No.	NR_1R_2
20, 37		**21, 38**	
22, 39		**23, 40**	
24, 41		**25, 42**	
26, 43		**27, 44**	
28, 45		**29, 46**	
30, 47		**31, 48**	
32, 49		**33, 50**	
34, 51		**35, 52**	
36, 53			

4.2.1.1 Biological evaluation of functionalized amino acid derivatives

Series 2, Scheme 3) (20-53):

Various concentrations of functionalized amino acid derivatives (**20-53**) were screened for cytotoxic activity on earlier defined nine human tumor and one non-tumorous cell lines.

Briefly, a three day MTT *in vitro* cytotoxicity assay was performed, which is based on the principle of uptake of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), a tetrazolium salt, by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically.[101] MTT was dissolved in phosphate buffered saline with a pH of 7.4 to obtain an MTT concentration of 5 mg/mL; the resulting mixture was filtered through a 0.22-micron filter to sterilize and remove a small amount of insoluble residue. For each type of tumor and normal cell, 5000 to 10,000 cells were seeded in a 96-well culture plate and incubated with various concentrations of functionalised amino acid derivatives (**20-53**) in a CO_2 incubator for 72 hours. Control cells not treated with functionalized amino acid derivatives (**20-53**) were similarly incubated. The assay was terminated after 72 hours by adding 125 μg (25 μL) of MTT to each well, then incubating for additional one hour, and finally adding 50 μL of 10% SDS-0.01N HCl to each well to lyse the cells and dissolve formazan. After incubating for one hour, the plate was read spectrophotometrically at 540 nm and the cytotoxicity percentage calculated using the following formula: Cytotoxicity percentage = 100 x $[1-(X/R_1)]$, where X = (absorbance of treated sample at 540 nm)-(absorbance of blank at 540 nm), R_1 = absorbance of control sample at 540 nm.

4.2.1.2 Structure activity relationship (SAR):

IC_{50} values were determined in micro molar (μM) concentrations of functionalized amino acid derivatives (**20-53**) for their *in vitro* cytotoxicity on three human cancer cell lines and one non-tumorous cell line. The human tumor cell lines used in the screening are ovary (PA-1), prostate (DU-145), oral (KB). The normal mouse fibroblast (NIH3T3) cell line was used to evaluate their cancer cell specificity (safety index). The cytotoxicity data is summarized in Table 4. Structure activity relationship (SAR) of these derivatives has been described below. In the present

discussion, compounds having IC_{50} > 10, 10-20 and > 20 μM have been designated as high, moderate and low cytotoxic derivatives, respectively.

Table 4: *In vitro* cytotoxicity data of functionalized amino acid derivatives (**20-53**)

Compound No.	IC_{50} (μM)			
	PA-1 (Ovary)	DU-145 (Prostate)	KB (Oral)	NIH3T3 (Normal fibroblast)
20	41.27	>100	45.70	NA
21	>100	>100	46.73	NA
22	>100	>100	24.89	NA
23	22.56	62.9	>100	74.5
24	9.41	64.41	>100	NA
25	5.67	27.75	12.6	86.6
26	85.17	>100	8.31	NA
27	13.13	87.72	16.12	NA
28	24.14	>100	>100	NA
29	6.1	24.34	4.17	26.2
30	89.32	>100	49.33	NA
31	37.46	69.59	>100	NA
32	>100	34.15	54.88	NA
33	>100	>100	>100	>100
34	57.32	>100	>100	NA
35	81.04	>100	47.88	NA
36	>100	>100	95.16	NA
40	>100	>100	22.02	NA
42	>100	>100	50.71	NA
43	69.53	25.9	12.5	NA
44	>100	>100	36.01	NA
45	56.9	84.06	36	NA
46	>100	>100	35.47	NA
48	89.54	>100	98.68	NA

49	>100	>100	37.82	NA
50	>100	>100	47.73	NA
51	>100	>100	96	NA
52	>100	>100	71.14	NA
53	>100	>100	>100	>100

NA = 'Not active

On ovary cancer cell line (PA-1) *N*-alkyl/ cycloalkyl groups showed weak activity but compound **23** having cyclohexyl substitutent exhibited better activity amongst these compounds. In *N*-aryl oxazolindine-5-carboxamide (**24-28**), compounds **24** and **25** showed potent activity IC_{50} of 9.41 μM and 5.67 μM, respectively, with good safety index > 10. Benzyl substituted oxazolindine-5-carboxamide derivative **29** showed potent inhibition with IC_{50} = 6.1 μM and safety index > 4. The heteroaryl substituents such as 2, 3 and 4-aminopyridine derivatiyes **30-32** showed low order activity. In the similar manner other substituents such as thiazole and cyclic amine containing compounds **33-36** were also exhibited low cytotoxicity. The opening of the substituted oxazolindine ring to produce corresponding amino alcohol derivatives resulted in the loss of activity on ovary cancer cell line.

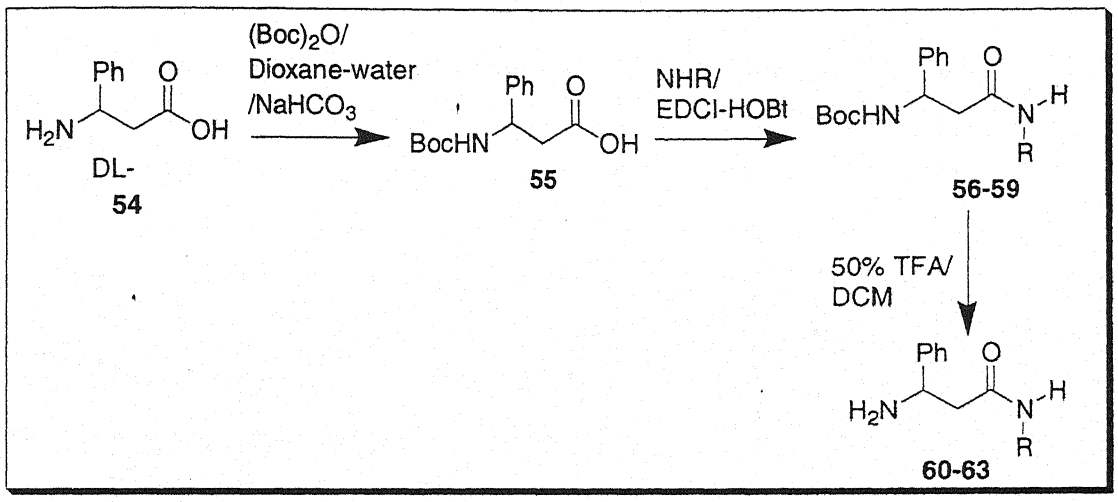
Oxazolindine and their corresponding amino alcohols showed the moderate to low order,cytotoxicity against prostate cancer cell line (DU-145).

Interesting results for these compounds were obtained against oral cancer cell line (KB), *N*-alkyl/ cycloalkyl derivatives exhibited low order cytotoxicity, whereas in the aryl substituted compounds **24-28**, the compound **26** with electron withdrawing group (cyano) showed high order cytotoxicity of IC_{50} = 8.31 μM with safety index > 10. The maximum cytotoxicity on this cancer cell line was shown by compound **29** with IC_{50} = 4.17 μM and safety index > 6. The ring opening of compound **26** into functionalized amino alcohol **43** also showed moderate cytotoxicity of IC_{50} = 12.5 μM.

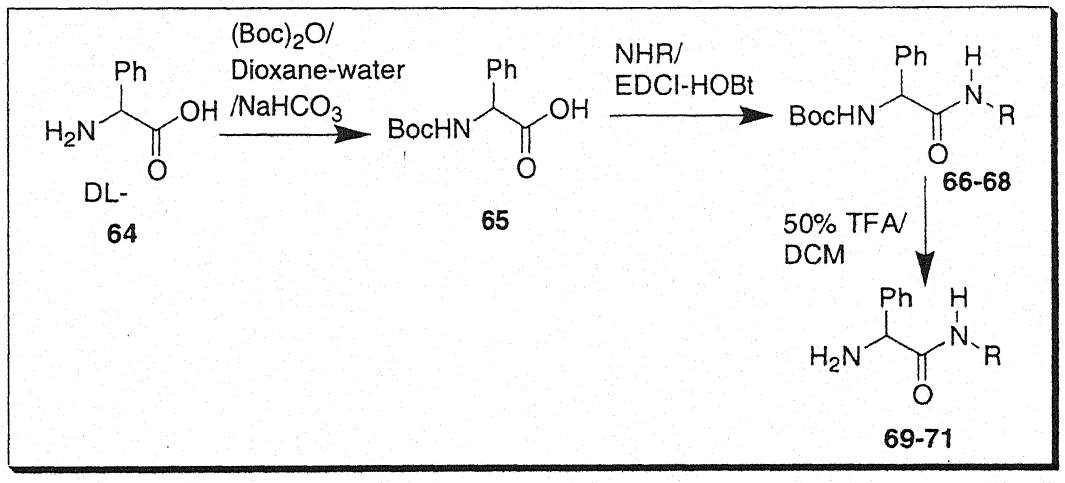
These results clearly indicated that oxazolindines (**20-36**), in general, were found superior than their corresponding amino alcohol derivatives (**37-53**). Amongst oxazolindine-5-carboxamide *N*-aryl (**24-28**) or *N*-benzyl (**29**) substituted compounds exhibited high cytototoxity against ovary and oral cancers with good safety profile.

4.2.1.3 Synthesis and characterization of functionalized amino acid derivatives (Series 3 and 4) (Scheme 4 and 5) (60-63 and 69-71):

Scheme 4: Synthesis of DL-3-amino-3-phenyl propionic acid derivatives (60-63)



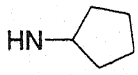
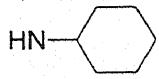
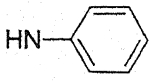
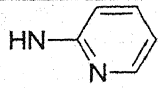
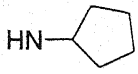
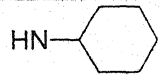
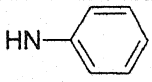
Scheme 5: Synthesis of DL-*N*-substituted phenyl glycinamide derivatives (69-71)



Synthesis of functionalized amino acid derivatives **60-63** and **69-71** are described in Scheme 4 and 5, respectively. The amino group of DL-3-amino-3-phenyl propionic acid **54** was protected with Boc anhydride to furnish Boc substituted amino acid **55**. Formation of Boc protected intermediate (**55**) was confirmed by comparison of melting point with the standard sample. The coupling of **55** with appropriate amine, using EDCI-HOBt provided the respective propionamide **56-59** and the Boc group of the later was removed by its treatment with 50% TFA/DCM to yield the corresponding DL-*N*-substituted 3-amino-3-phenyl propionamide (**60-63**). Similarly,

DL-*N*-substituted phenyl glycinamide derivatives (**69-71**) were prepared starting from DL-phenyl glycine (**64**). Each compound was characterized by ^{1}H NMR and EIMS studies. The structures of the compounds are shown in Table 5.

Table-5: Functionalized amino acid derivatives (**56-63** and **66-71**)

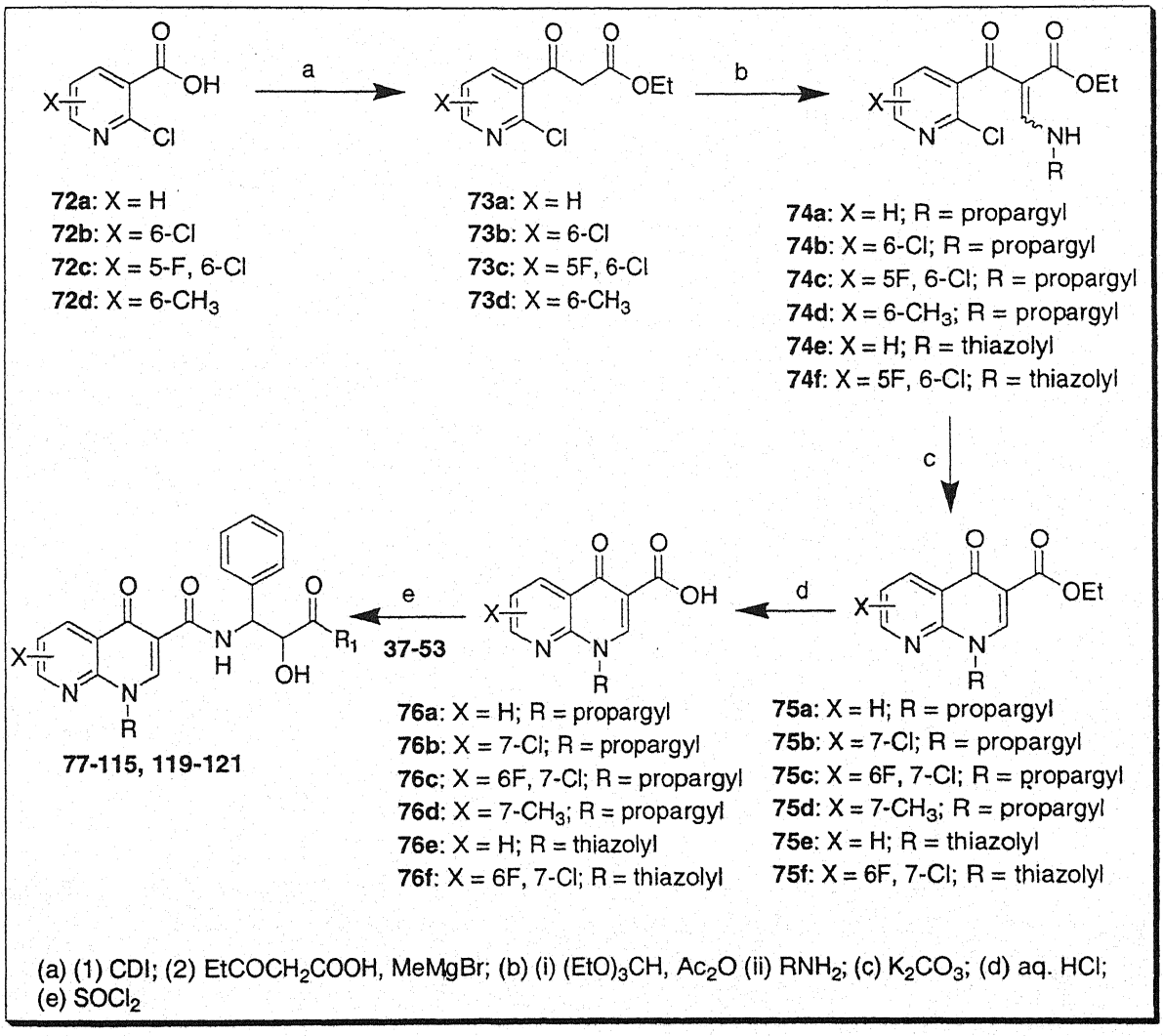
Compound No.	R	Compound No.	R
56, 60		**57, 61**	
58, 62		**59, 63**	
66, 69		**67, 70**	
68, 71		-	-

4.2.2 Synthesis and characterization of 1,8-naphthyridine-3-carboxamide derivatives

4.2.2.1 Synthesis and characterization of 1,8-naphthyridine-3-carboxamide derivatives (Series 5) (Scheme 6 and 7):

Synthesis of substituted/ unsubstituted 1,8-naphthyridine-3-carboxamide derivatives (**77-115** and **119-121**) have been described in Scheme 6 and 7.

Scheme 6: Synthesis of 1,8-naphthyridine-3-carboxamide derivatives



Commercially available, nicotinic acid **72a-d** was reacted with 1,1'-carbonyldiimidazole (CDI) in dry THF to result the imidazolide solution, which was allowed to react with ethyl hydrogen malonate and methyl magnesium bromide to afford nicotinoylacetate **73a-d**.

Compounds **73a-d** were identified on the basis of ^{1}H NMR and EIMS spectral data. The ^{1}H NMR spectra showed characteristic peaks for the aromatic proton. A multiplet at δ 4.08-4.32 ppm for $-OCH_2$ protons and triplet at δ 1.21-1.36 ppm for $-CH_3$ protons has been observed in case of unsubstituted nicotinoylacetate (**73a**). The EIMS spectra of **73a** showed molecular ion peak at m/z (relative intensity) 226.3. In addition isotopic peak for C-2 ^{37}Cl appears at m/z (relative intensity) 228.3 (25%). C-6 methyl substituted 1,8-naphthyridine-3-carboxamide derivative **73d** showed peak for the methyl proton at 2.55 ppm. Molecular ion peaks for 2,6-dichloro and 2,6-dichloro-5-fluoro substituted nicotinylacetate **73b** and **73c** were observed at 261 and 279, respectively.

Compounds **73a-d**, on treatment with triethyl orthoformate and acetic anhydride followed by the addition of primary amines (2-aminothiazole and propargyl amine) to afford ethyl nicotinoylacrylate **74a-f**. Compounds **74a-f** were used as such for the next step in crude form with out further purification.

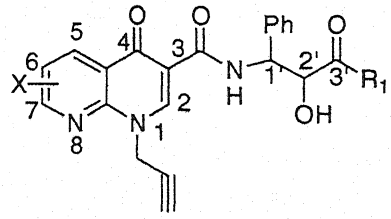
Ethyl 1,8-naphthyridine-3-carboxylate (**75a-f**) were prepared by base-assisted (K_2CO_3) cyclization of acrylate **74a-f** in ethyl acetate as a solvent. In case of unsubstituted ethylester of 1-propargyl-1,8-naphthyridine carboxylic acid (**75a**), aromatic protons were observed in region of 7.41-8.92 as multiplet, triplet was observed for CH_3 at 1.42 ppm with coupling constant of 7.0 Hz. Methylene proton of ester showed a quartet with coupling constant of 7.0 Hz. N-1 propargyl alkyne proton appeared at δ of 2.5 ppm in $CDCl_3$ solution but it get shifted to 2.75 in mixture of $CDCl_3$ and DMSO-d_6 solution. While, in the neat DMSO-d_6 solution, it was shifted upto 3.5 ppm, which is due to the deshielding effect of the DMSO-d_6 solution. NCH_2 proton showed a singlet at 5.23 ppm. In case of 2-amino thiazole substituted ethylester (**75e** and **75f**) of 1,8-naphthyridine carboxylic acid, two more protons were appeared in aromatic region.

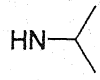
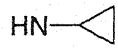
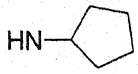
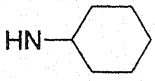
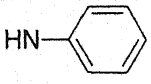
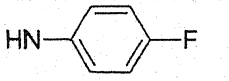
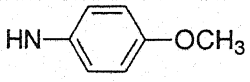
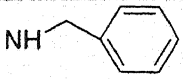
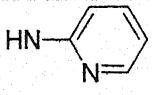
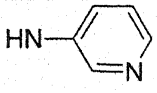
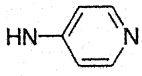
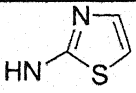
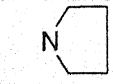
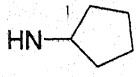
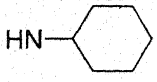
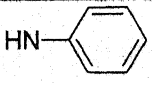
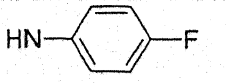
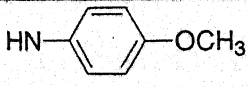
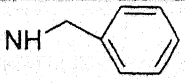
Ethyl 1,8-naphthyridine-3-carboxylate (**75a-f**) upon acidic hydrolysis provided 1,8-naphthyridine-3-carboxylic acid (**76a-f**). ^{1}HNMR peaks for the ethyl part got disappeared and a broad singlet appeared at δ of 14.26 ppm for O<u>H</u> proton in unsubstituted 1,8-naphthyridine-3-carboxylic acid **76a**.

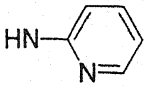
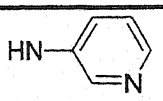
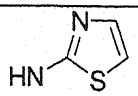
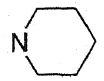
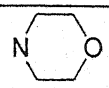
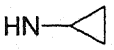
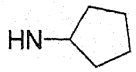
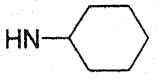
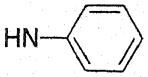
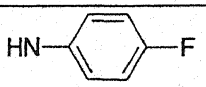
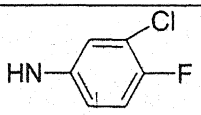
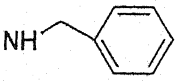
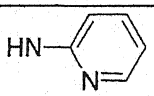
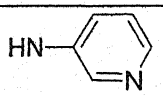
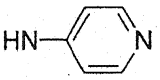
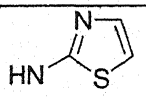
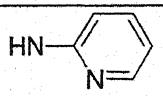
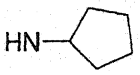
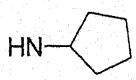
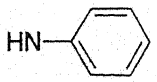
Compounds **76a-f** were treated with thionyl chloride to afford their acyl chloride intermediate, respectively. The 1,8-naphthyridine-3-carboxamide derivatives (**77-115** and **119-121**) were prepared by coupling of 1,8-naphthyridine-3-carbonyl

chloride intermediate with their functionalized amino acids (**37-53**). The 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives **77-118** are listed in Table 6.

Table-6: 1,8-Naphthyridine-3-carboxamide derivatives (**77-118**)



Compound No.	R_1	X	Compound No.	R_1	X
77		H	**78**		H
79		H	**80**		H
81		H	**82**		H
83		H	**84**		H
85		H	**86**		H
87		H	**88**		H
89		H	**90**		H
91		7-Cl	**92**		7-Cl
93		7-Cl	**94**		7-Cl
95		7-Cl	**96**		7-Cl

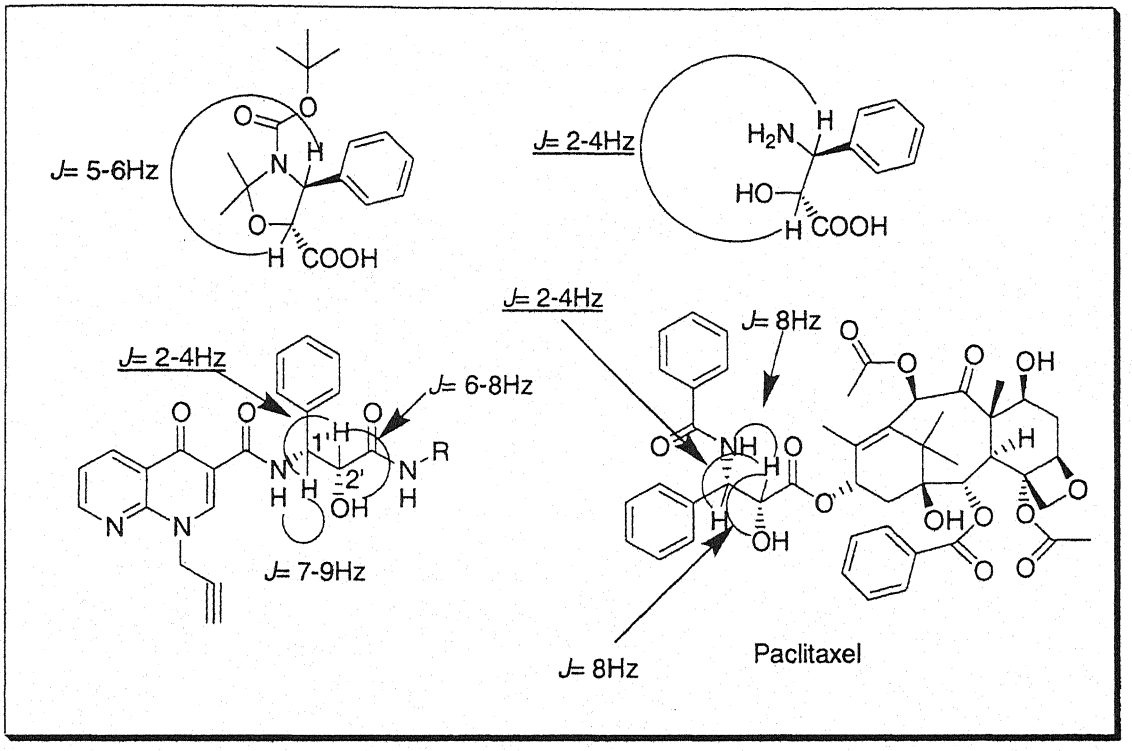
Compound No.	R_1	X	Compound No.	R_1	X
97		7-Cl	**98**		7-Cl
99		7-Cl	**100**		7-Cl
101		7-Cl	**102**		6-F, 7-Cl
103		6-F, 7-Cl	**104**		6-F, 7-Cl
105		6-F, 7-Cl	**106**		6-F, 7-Cl
107		6-F, 7-Cl	**108**		6-F, 7-Cl
109		6-F, 7-Cl	**110**		6-F, 7-Cl
111		6-F, 7-Cl	**112**		6-F, 7-Cl
113		6-F, 7-Cl	**114**		$7\text{-}CH_3$
115	OCH_2CH_3	H	**116**		6-F, 7-pyrrolidine
117		6-F, 7-(3"-methylpiperidine)	**118**		6-F, 7-(3"-methylpiperidine)

The coupling constant here in between the proton at C-1'and C-2' remains constant, which has showed that no change in the stereochemistry of proton has took place and can be correlated to side chain of paclitaxel[102] as shown in Figure 1.

Compound **103** on treatment with pyrrolidine and 3'-methyl piperidine in the presence of triethylamine yielded compound **116** and **117**, respectively. Under the

similar conditions, compound **105** on treatment with 3'-methyl piperidine afforded compound **118**, as shown in Scheme 7. The 2-aminothiazolyl-1,8-naphthyridine-3-carboxamide derivatives **119-121** are listed in Table 7.

Figure 1: Comparison of coupling constant



Scheme 7: Synthesis of 1,8-naphthyridine-3-carboxamide derivatives

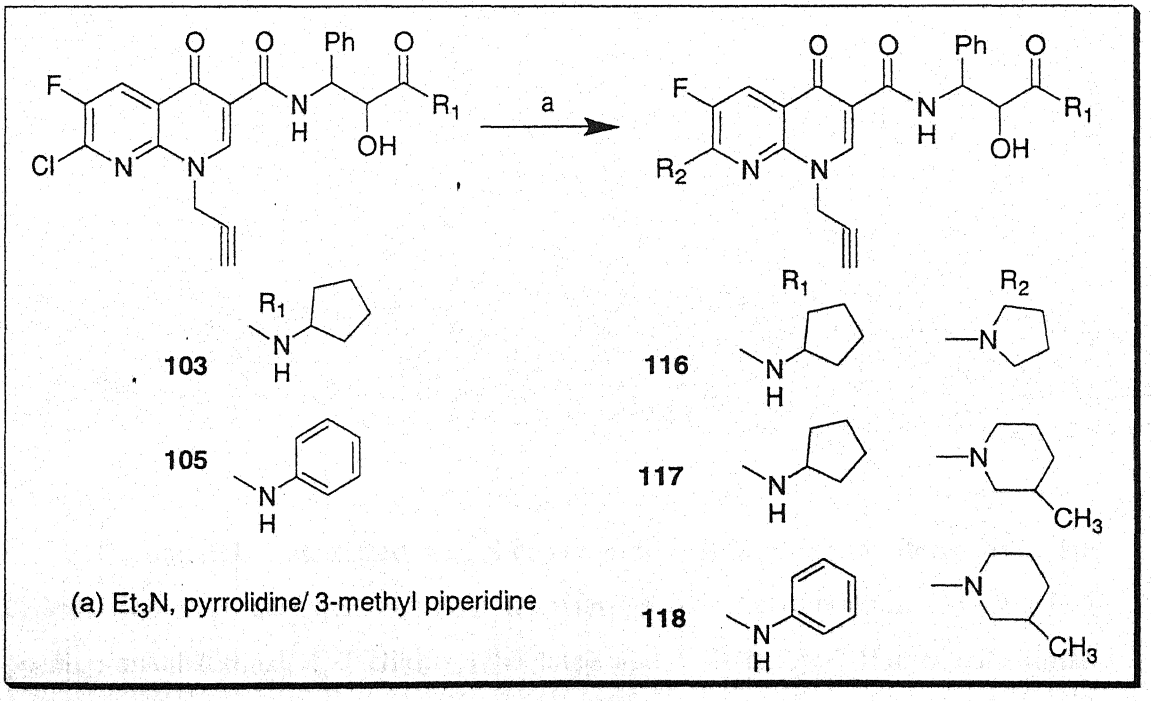
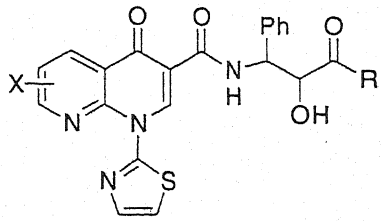
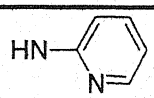
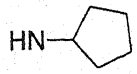


Table-7: 1,8-Naphthyridine-3-carboxamide derivatives (**119-121**)



Compound No.	R	X	Compound No.	R	X
119		H	**120**		6-F, 7-Cl
121		6-F, 7-Cl	-	-	-

4.2.2.1.1 Structure activity relationship (SAR):

All the synthesized 1,8-naphthyridine-3-carboxamide derivatives (**77-121**) were tested for *in vitro* cytotoxicity on nine tumor as well as a non-tumorous cell lines and IC_{50} values were calculated in micro mole (μM). The human tumor cell lines used in the study are ovary (PA1), prostate (DU145), oral (KB), colon (SW620), breast (HBL100), lung (A-549), pancreas (MIAPaCa2), leukemia (K562) and endothelial (ECV304) cancers. Compounds (**77-121**) were also screened against normal mouse fibroblast (NIH3T3) cell line to evaluate their cancer cell specificity (safety index). Derivatives of 1,8-naphthyridine (**77-121**) were screened for cytotoxic activity at the highest soluble concentration of 10 μM and on four lower concentrations on nine human tumor and one non-tumorous cell lines. The cytotoxicity data is summarized in Table 8. Compounds, which were found inactive at 10 μM, are not listed in Table 8. In 1,8-naphthyridine-3-carboxamide derivatives 3-*N*-(3'-*N*'-substituted-2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1,8-naphthyridine-3-carboxamide derivatives, substitution in 1,8-naphthyridine ring had played crucial role in eliciting the activity.

Compounds synthesized in 1,8-naphthyridine-3-carboxamide derivatives are divided into three categories (compounds with out any substitution at C-6 and C-7 position-unsubstituted, C-7 chloro substituted and C-6 chloro-7-fluoro substituted compounds) based on the substitution pattern at C-6 and C-7 position.

Table 8: *In vitro* cytotoxicity of 1,8-Naphthyridine-3-carboxamide derivatives (**77-121**)

Compd. No	IC_{50} (μM)									
	PA-1 (Ovary)	DU-145 (Prostate)	KB (Oral)	SW620 (Colon)	HBL100 (Breast)	A549 (Lung)	Miapaca (Pancreas)	K-562 (Leukemia)	NIH3T3 (Normal fibroblast)	CHO (Normal ovary)
81*	9.1	2.9	>10	>10	5.9	6.09	9.81	>10	4.79	NA
85**	0.41	>10	3.7	1.4	4.1	3.06	>10	4.4	2.2	NA
87	9.9	>10	>10	>10	>10	>10	>10	>10	NA	NA
91	3.12	>10	>10	6.2	8.99	>10	8.26	6.34	9.76	NA
92	1.19	>10	>10	4.62	4.7	9	8	9.4	5	8.7
94	1.2	6.1	2.6	3.2	6.9	>10	>10	>10	NA	NA
95	3.22	>10	>10	5.27	8.8	>10	8.2	>10	7.3	NA
96	8.1	1.6	>10	>10	>10	>10	>10	>10	NA	NA
97	7.6	>10	>10	>10	>10	>10	9.0	>10	NA	NA
98	2.0	>10	>10	7.8	7.5	>10	7.3	>10	5.5	NA
99	3.49	>10	>10	7.13	4	9	7.6	9.2	1.8	9.7
100	3.33	>10	>10	>10	>10	>10	>10	>10	NA	NA
101	3.95	>10	>10	>10	9.1	>10	9.1	>10	NA	NA
102	2.54	8.56	9.6	4.11	6.60	>10	>10	8.60	5.86	NA
103	3.55	>10	>10	2.79	>10	1.5	0.41	0.77	1.05	NA
104	1.7	>10	7.2	4.4	5.0	6.8	3.8	9.9	3.4	2.2
105	>10	>10	>10	>10	4.33	4.82	1.28	2.50	2.24	NA
107	3.6	6.1	3.5	2.8	8.2	9.9	6.7	>10	7.9	2.4
108	>10	>10	>10	>10	9.26	6.99	3.17	5.39	2.85	NA
109	2.62	3.45	9.12	3.79	3.17	9.53	2.83	8.20	4.86	NA
110	3.1	>10	>10	7.9	8.7	>10	3.3	8.4	4.1	NA
111	4.7	>10	8.42	3.35	5.5	>10	8.1	7	7.6	NA
112	3.1	3.6	7.9	6.3	3.20	2.58	3.50	4.16	5.31	NA
113	>10	>10	>10	>10	5.22	7.49	1.78	4.99	1.69	NA
120	1.4	>10	2.5	2.3	2.5	6.7	3.7	5.2	3.7	5.4

Compd. No	IC_{50} (μM)									
	PA-1 (Ovary)	DU-145 (Prostate)	KB (Oral)	SW620 (Colon)	HBL100 (Breast)	A549 (Lung)	Miapaca (Pancreas)	K-562 (Leukemia)	NIH3T3 (Normal fibroblast)	CHO (Normal ovary)
121	>10	>10	>10	>10	7.1	5.7	3.8	8.0	NA	1.6

*** Salt; * Precipitation observed during aqueous dilution; NA = 'Not active' on more than 10 μM concentration*

Unsubstituted 3-*N*-(3'-*N*'-alkyl-2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**77-80**) were found inactive. The C-7-chloro-3-*N*-(3'-*N*'-alkyl-2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**91** and **92**) have showed improved cytotoxicity than unsubstituted one. The cyclohexyl-substituted derivative **92** has exhibited potent cytotoxicity on ovarian cancer cell line (PA-1) with IC_{50} of 1.19 μM and high safety index of 7.3. The C-6-chloro-7-fluoro-3-*N*-(3'-*N*'-alkyl-2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**102-104**) showed potent to moderate activity on ovarian and other cancer cell lines. The cyclopentyl substituted (**103**) is the highly potent compound in this Series which, has shown IC_{50} of 0.41 and 0.77 μM on pancreas (Miapaca) and leukemia (K-652) cancer cell lines, respectively.

In 3-*N*-(3'-*N*'-aryl-2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives in unsubstituted derivatives (**81-84**), compound **81** has shown IC_{50} of 2.9 μM on prostate (DU-145) cancer cell line, while the other derivatives were inactive. In C-7-chloro-3-*N*-(3'-*N*'-aryl-2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**93-95**), compound **95** with electron donating methoxy group has showed moderate to low activity. *N*'-benzyl substituted derivative **96** has shown selective cytotoxicity on prostate (DU145) cancer cell line with IC_{50} of 1.6 μM. In C-7-chloro-C-6-fluoro-3-*N*-(3'- *N*'-aryl -2'- hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**105-107**), compound **105** showed potent activity on pancreas (Miapaca) cell line with IC_{50} of

1.28 μM. While, compound **107** and benzyl substituted derivative **108** has shown moderate to low activity.

In 3-*N*-(3'-*N*'-heteroaryl-2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives, unsubstituted derivatives (**85-88**), compound **85** is the most potent compound of this Series with IC_{50} of 0.41 and 1.4 μM on ovary (PA-1) and colon (SW620) cancer cell lines, respectively. Changing the position of the nitrogen in pyridine ring leads to the loss of activity (**86** and **87**).

In C-7-chloro-3-*N*-(3'-*N*'-heteroaryl-2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**97-99**), 3'-pyridine derivative compound **98** has showed potent activity on ovary (PA-1) cancer cell line with IC_{50} of 2.0 μM, while, 2-aminothiazole derivative (**99**) has showed moderate to low activity on other cancer cell lines. In C-7-chloro-C-6-fluoro-3-*N*-(3'- *N*'-heteroaryl-2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**109-112**), all compounds showed potent activity on ovary (PA-1) cancer cell line with $IC_{50} < 4.7$ μM. 2'-Pyridine substituted derivative (**109**) has showed $IC_{50} < 5.0$ μM on six cancer cell lines.

In *N*-3'-tertiary amine substituted 3-*N*-(2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives, unsubstituted derivatives (**89** and **90**) were inactive. In C-7-chloro-*N*-3'-tertiary amine substituted 3-*N*-(2'-hydroxy-1'-phenyl propane-3'-carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives piperidine (**100**) and morpholino (**101**) showed selectivity towards ovary (PA-1) cancer cell line. C-7-Chloro-C-6-fluoro- 3-*N*- (3'-*N*'- piperidine - 2'- hydroxy- 1'- phenylpropane -3'- carboxamide)-1-propargyl-1,8-naphthyridine-3-carboxamide (**113**) has shown potent activity on pancreas (Miapaca) cell line and modest to low activities on other cell lines.

Substitution of C-7 position with group having inductive effect like methyl (**114**) leads to the complete loss of activity. Replacement of the C-3' amide group by ester (**115**) linkage caused complete loss of activity.

Modifications were carried out at C-7 position in potent 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**103** and **105**) by treating with different secondary amines to increase the selectivity and potency of C-6 chloro-C-7 fluoro potent derivatives as shown in Scheme 3. In compound **103**, substitution of C-7 chloro by pyrrolidine (**116**) and 3''-methylpiperidine ring (**117**) caused complete loss

of activity. Similarly, in compound **105**, substitution of C-7 chloro by 3''-methylpiperidine ring (**118**) leads to inactive compound. This indicates that C-7 halo is essential for the activity.

Based on the activity profile of propargyl compounds (**85**, **103** and **113**), some 2-thiazolyl derivatives were synthesized (**119-121**) as shown in Table 7. 2-Thiazolyl derivative (**120**) of compound **103** has showed two times more selectivity towards ovary cancer cell line but selectivity towards pancreas (Miapaca) and leukemia (K-562) has decreased. While, in case of compound (**119**) activity was completely lost. Similarly, compound (**121**) has not showed significant change on replacement of the propargyl group.

The results clearly indicated that compounds bearing propargyl substituent at N-1 showed potent activity in comparison to other groups might be due to the acidic nature of the acetylene hydrogen. C-7 Chloro group has played an essential role in C-6 chloro and C-7 fluoro substituted compounds. Replacement of the C-7 chloro with other groups caused the complete loss in activity in this Series. Amongst 1,8-naphthyridine-3-carboxamide derivatives, compounds **81**, **85**, **103** and **105** has showed very potent activity on different cancer cell lines.

4.2.2.1.2 Anti-inflammatory activity of 1,8-naphthyridine-3-carboxamide derivatives (Series 5) (Scheme 6 and 7) (77-121):

Compounds of Series 5 have shown promising anticancer activities and were further tested for their potential anti-inflammatory activity based on the molecular link between cancer and inflammation. Compounds **105**, **107**, **108**, **112** and **113** and **120** exhibited a very high TNF-α inhibition at 1 μg/ml. Table 9 demonstrates IC_{50} value for TNF-α inhibition by selected molecules screened at various concentrations ranging from 0.001to10 μg/ml.

Table 9: IC_{50} values for TNF-α modulation by selected 1,8-naphthyridine carboxamide derivatives

Compound No.	IC_{50} value (μg)
97	<0.001
100	<0.001
101	0.31
104	<0.001
106	~0.001
109	<0.001
110	1.1
111	0.59

Compounds showing high TNF-α down regulation **105**, **107**, **108**, **113** and **120** were also found to be potent inhibitors of IL-1-β secretion by LPS-stimulated DCs. Inhibition of MIP-1-α and IP-10 (pro-inflammatory chemokines) activity is suggestive of significant anti-inflammatory activity of 1,8-naphthyridine-3-carboxamide derivatives. Compounds **100**, **101**, **105**, **106**, **109** and **111** showed >50% down regulation of MIP-1-α in addition to TNF-α and IL-1-β inhibition. Compounds **101**, **104**, **111** and **112** have demonstrated high IP-10 inhibitory activity as shown in Table 10.

Table 10: Down regulation of IP-10 levels to 50% (IC_{50}) of selected 1,8-naphthyridine derivatives

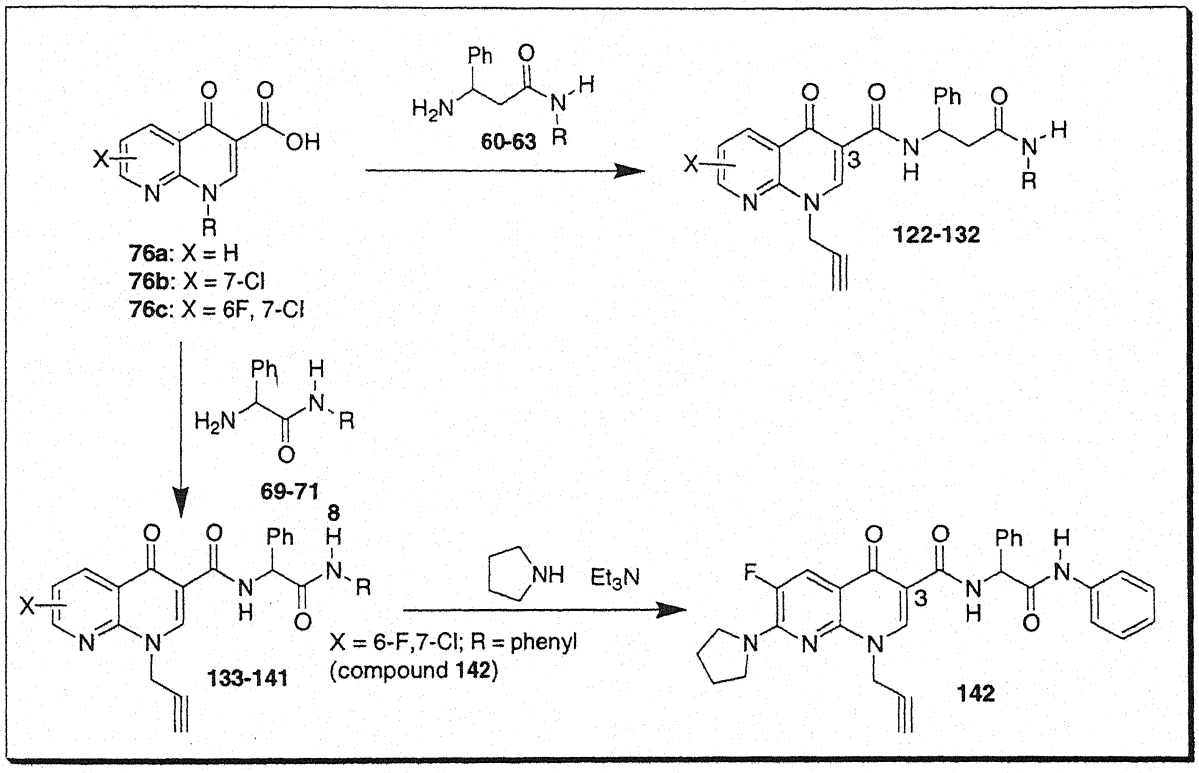
Compound No.	IC_{50} value (μg)
101	0.32
110	1.1
111	0.62

Compounds **101** and **111** were able to induce remarkable down regulation of TNF-α, IL-1-β, MIP-1-α and IP-10 activity and hence were found to be most active anti-inflammatory compounds among 1,8-naphthyridine-3-carboxamide derivatives.

4.2.2.2 Synthesis and characterization of 1,8-naphthyridine-3-carboxamide derivatives (Series 5) (Scheme 8):

Based on the structure activity relationship of Series 5 (Scheme 6 and 7), to further enhance the activity of *N*-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives, we had substituted the carboxylic part with different amino groups of Series 3 and 4. Synthesis of compounds **122-142** has been confirmed by ^{1}H NMR and EIMS studies. Particularly, ^{1}H NMR of compounds **122-132** has showed multiplet for methylene proton of amino acid side chain in region of δ of 2-3 ppm.

Scheme 8: Synthesis of 1,8-naphthyridine-3-carboxamide derivatives

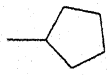
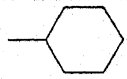
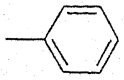
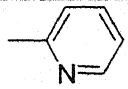
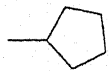
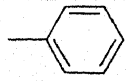
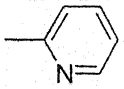
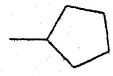
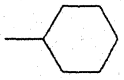
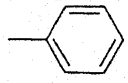
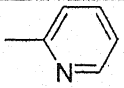
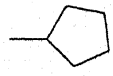
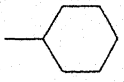
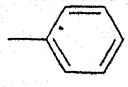
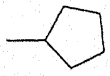
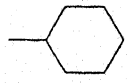
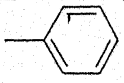
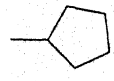
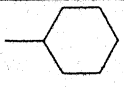
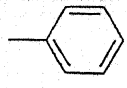
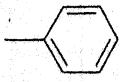


4.2.2.2.1 Structure activity relationship (SAR):

Amongst 3-(*N*-substituted phenylpropionamide)-1-propargyl-1,8-naphthyridine carboxamides (**122-142**), substitution in 1,8-naphthyridine ring had played crucial role in eliciting cytotoxicity. The compounds are shown in Table 11. Unsubstituted 1,8-naphthyridine derivatives (**122-125**) were found inactive except compound **124**, which showed cytotoxicity on prostate cancer cell line. The C-6 and C-7 halo substituted 1,8-naphthyridine derivatives (**126-132**) were found better than unsubstituted ones (**122-125**). The entire halo substituted derivatives, except

compound **132**, had exhibited potent (IC_{50} < 4 μM) cytotoxicity against ovarian cancer cell line. The 3-(*N*-3'-cyclopentyl phenylpropionamide)-1-propargyl-1,8-naphthyridine carboxamide (**126**) and its 6-fluoro-7-chloro-1,8-naphthyridine analog **129**, have shown potent cytotoxicity against ovarian, colon and breast cancer cell lines.

Table-11: List of 1-propargyl-1,8-naphthyridine derivatives (Series 5) (Scheme 8) (122-142):

Compd. No.	X	R	Compd. No.	X	R
122	H		**123**	H	
124	H		**125**	H	
126	7-Cl		**127**	7-Cl	
128	7-Cl		**129**	6-F,7-Cl	
130	6-F,7-Cl		**131**	6-F,7-Cl	
132	6-F,7-Cl		**133**	H	
134	H		**135**	H	
136	7-Cl		**137**	7-Cl	
138	7-Cl		**139**	6-F,7-Cl	
140	6-F,7-Cl		**141**	6-F,7-Cl	
142	6-F, 7-pyrrolidine		-	-	-

Compound **126** had shown relatively better safety index than compound **129**. In addition, compound **126** also elicited potent cytotoxicity against pancreas cancer cell

lines, while compound **129** was found as a most potent (IC50 2.0μM) derivative against breast cancer cell line. Upon expansion of cyclopentyl ring in compound **129** to cyclohexyl ring (compound **130**), cytotoxicity was lowered, though compound **130** showed potent cytotoxicity against ovarian cancer cell line. The 7-chloro-3-(*N*-3'-phenyl phenylpropionamide)-1-propargyl- 1,8- naphthyridine carboxamide (**127**) and its 6-fluoro-7-chloro-1,8- naphthyridine analog **131** exhibited potent cytotoxicity on ovarian cancer cell line with safety index of > 2. In addition, compound **127** also elicited potent activity on colon, breast, pancreas and endothelial cancer cell lines but with safety index (< 2).

Table 12: In vitro cytotoxicity of 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (Series 5) (Scheme 8) (122-142):

Comp No.	IC_{50} (μM)									
	PA-1 (Ovary)	DU-145 (Prostate)	KB (Oral)	SW620 (Colon)	HBL100 (Breast)	A549 (Lung)	Miapaca (Pancreas)	K-562 (Leukemia	ECV304 (Endotheial)	NIH3T3 (Normal fibroblast)
124	>10	7.2	>10	>10	>10	>10	>10	>10	>10	NA
126	1.1	>10	>10	2.7	3.2	9.5	2.4	6.8	7.8	4.4
127	0.54	>10	>10	2.9	4.0	>10	3.0	7.7	4.0	4.4
128	1.7	4.9	5.2	2.9	>10	>10	>10	5.9	>10	NA
129	0.68	>10	4.9	2.1	2.0	6.1	4.4	7.3	5.1	2.4
130	2.3	8.03	>10	>10	>10	>10	4.9	6.2	9.8	NA
131	2.1	5.9	>10	5.7	9.3	>10	6.9	6.6	>10	9.7
132	>10	2.1	2.3	2.3	>10	>10	>10	>10	>10	4.2
133	>10	>10	4.5	>10	>10	>10	>10	>10	>10	NA
135	8.9	8.8	3.0	>10	>10	>10	>10	>10	>10	NA
138	>10	1.7	2.1	2.2	7.8	>10	9.7	3.3	>10	NA
139	1.8	3.2	3.5	3.4	5.1	>10	1.7	5.9	3.2	0.4
140	1.8	>10	>10	>10	>10	>10	>10	>10	>10	7.4
141	0.5	0.6	1.1	1.4	>10	>10	>10	5.0	9.1	NA

On the other hand, 7-chloro-3-(*N*-3'-(2''-pyridine) phenylpropionamide)-1-propargyl-1,8- naphthyridine carboxamide (**128**) exhibited potent cytotoxicity on ovarian cancer cell line and interestingly, its 6-fluoro-7-chloro-1,8- naphthyridine analog **132** was found inactive against the same cell line. However, both the derivatives have shown potent cytotoxicity on colon cancer cell line. In addition, compound **132** also showed potent activity against prostate and oral cancer cell lines with safety index of ~ 2. It indicated that the halo substitution at C-6 and C-7 position in 1,8-naphthyridine-3-carboxamides is essential for eliciting cytotoxicity. The 3-(phenylpropionamide)-1-propargyl- 1,8- naphthyridine carboxamides possessing cycloalkyl and phenyl substituents were found better choice for cytotoxicity against ovarian cancer cell line.

The 3-(*N*-phenylglycinamide)- 1- propargyl- 1,8- naphthyridine carboxamides (**133-135**) were found relatively better than their 3-(*N*-phenylpropionamide)- 1,8-naphthyridine carboxamide derivatives (**122-124**). The compound **135** having *N*-phenyl phenylglycinamide substituent at C-3 position, showed potent cytotoxicity against oral cancer cell line and compound **133** also showed cytotoxicity against oral cancer cell line while compound **134** was found inactive. Interestingly, the C-7-chloro-(*N*-phenylglycinamide)-1-propargyl-1,8- naphthyridine carboxamides **136** and **137** were found inactive. However, C-7-chloro-1,8-naphthyridine having *N*-phenyl phenylglycinamide substituent at C-3 position (**138**), exhibited potent cytotoxicity against prostate, oral, colon, and leukemia cancer cell lines along with high safety index. Additionally, compound **138** was identified as a most potent (IC_{50} 3.3 μM) derivative against leukemia cancer cell line. As discussed for 1,8-naphthyridine derivatives having *N*-phenylpropionamide substituent at C-3 (**129-131**), its 3-(*N*-phenylglycinamide) 1,8- naphthyridine derivatives (**139-141**) also showed potent cytotoxicity against a number of cancer cell lines. Compound **139** showed potent cytotoxicity against ovarian, prostate, oral, colon, pancreas and endothelial cancer cell lines but possessed safety index of < 2. Compound **140** was found selective as well as potent cytotoxic against ovarian cancer cell line along with safety index > 2. Compound **141** having *N*-phenyl phenylglycinamide substituent at C-3, in 1,8-naphthyridine was found as a most potent (IC_{50} 0.5-1.4 μM) derivative against ovarian, prostate, oral and colon cancer cell line along with high safety index. Upon replacement of the 7-chloro group in compound **141** with pyrrolidine (compound **142**), cytotoxicity was lost. It indicated that 1-propargyl - 1, 8- naphthyridine -3-

carboxamides have shown potent cytotoxicity on a number of cancer cell lines. The C-6 and C-7 halo substituent in the 1,8-naphthyridine played a crucial role in eliciting cytotoxicity. Compound **141** is selected for further studies.

Compounds **122**, **124**, **128**, **129**, **131**, **132** and **138** were found to demonstrate significant anti-inflammatory activity, reflected by >50% inhibition of IL-1-β at 1μg/ml and 0.1μg/ml. Compounds **125,126,127,128,129,131,132,135,138** and **141** were able to significantly downregulate IL-6 secretion by LPS stimulated DCs. Compound **131** was found to be most active as it was able to show a significant down regulation of TNF-α and IP-10 also in addition to IL-1-β and IL-6.

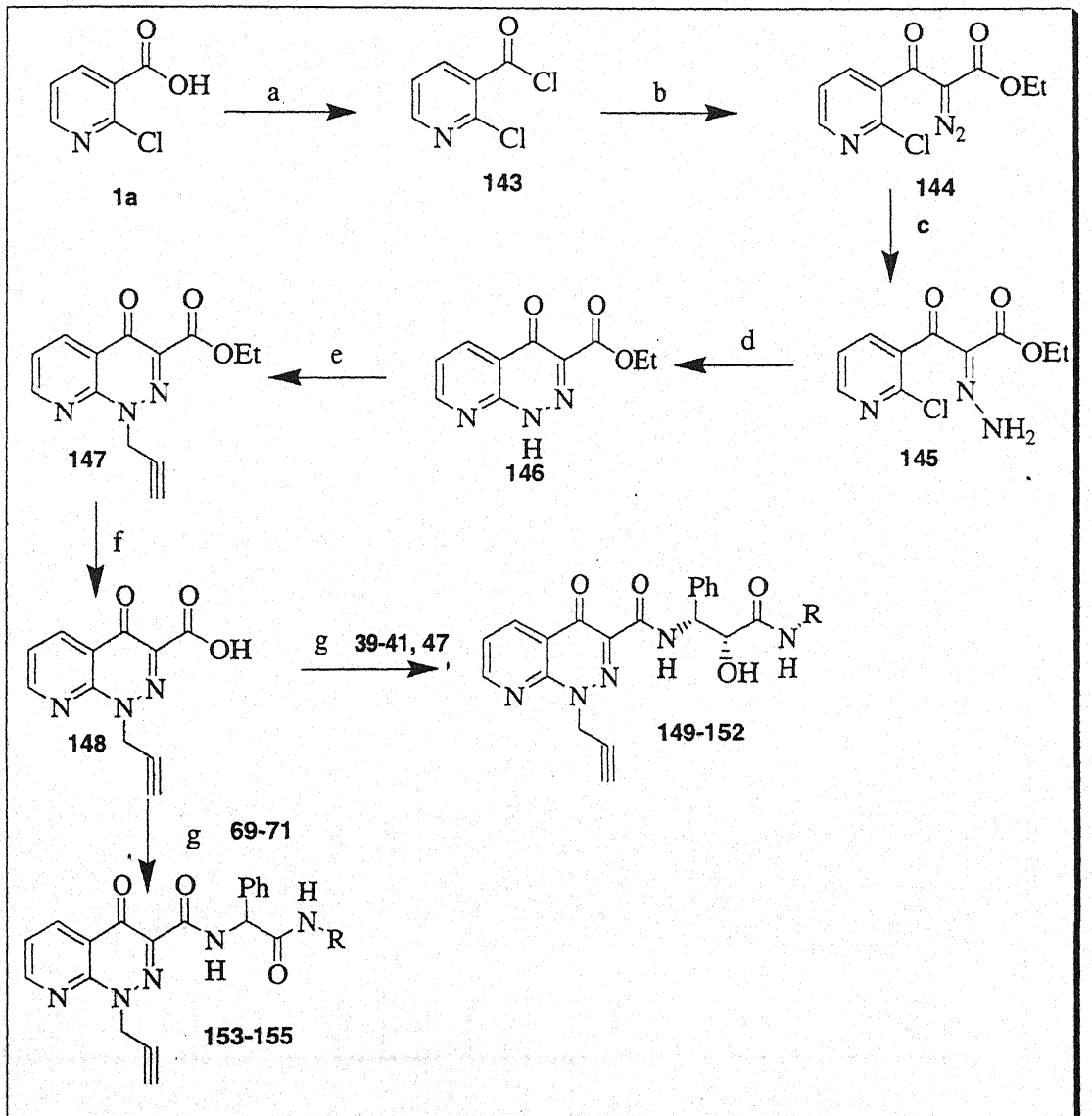
4.2.2.3 Synthesis and characterization of 1-propargyl-pyrido[2,3-*c*] pyridazine-3-carboxamide derivatives (Series 6) (Scheme 9):

To synthesize pyrido[2,3-c]pyridazine heterocyclic system nicotinic acid **1a** was refluxed with thionyl chloride to afford compound 2-chloronicotinoyl chloride (**143**) as shown in Scheme 9. Compound **143** was allowed to react with ethyl diazoacetate to provide 2-diazo nicotinoylacetate (**144**).[103] ^{1}H NMR of compound **144** showed three distinct peaks for the aromatic protons at δ 7.27-8.48 ppm. While, OCH_2 showed a characteristic quartet at 4.18 ppm and methyl protons split into a triplet at δ 1.17 ppm. Compound **144** was treated with triphenylphosphine in isopropyl ether and resulting 2-hydrazono nicotinoylacetate (**145**) was refluxed in mixture of methanol and water to afford ethyl pyrido[2,3-c]pyridazine-3-carboxylate (**146**). ^{1}H NMR of compound **146** showed a broad singlet for NH proton at δ 10.67 ppm along with characteristic peaks for OCH_2 and CH_3 protons. Ethyl-*N*-1-propargyl pyrido[2,3-c]pyridazine-3-carboxylate (**147**) was prepared by the N-alkylation of compound **146** in presence of NaH. ^{1}H NMR peak for the propargyl proton was obtained as triplet at δ 2.38 ppm and NCH_2 proton showed a doublet at 5.42 ppm. Compound **147** upon basic hydrolysis afforded *N*-1-propargyl pyrido[2,3-c]pyridazine-3-carboxylic acid (**148**). Compound **148** showed the absence of the peak for ethyl part and mass peak for it was observed at 228. The synthesis of *N*-1-propargyl pyrido[2,3-c]pyridazine-3-carboxamide derivatives (**149-155**) was carried out in a similar way to 1,8-naphthyridine-3-carboxamide derivatives Scheme 6. The structures of compounds are shown in Table 13.

4.2.2.3.1 Structure Activity Relationship (SAR):

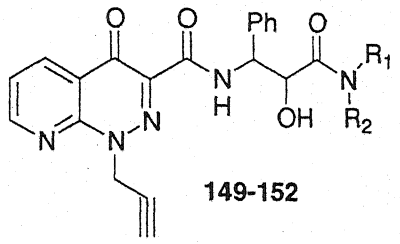
To further enhance the activity of 1,8-naphthyridine, we carried out the isosteric replacement of carbon at C-2 position of selected molecules with nitrogen to provide a new core structure of pyrido[2,3-*c*]pyridazine ring system. But the activity was decreased down. Compound **152** has showed moderate activity on A549 (lung) cancer cell line with IC_{50} of 6.0 μM. Compounds **154** and **155** have showed slight activity on KB (oral) cancer cell line. List of the active compounds is shown in Table 14.

Scheme 9: Synthesis of 1-Propargyl-pyrido[2,3-*c*] pyridazine-3-carboxamide derivatives

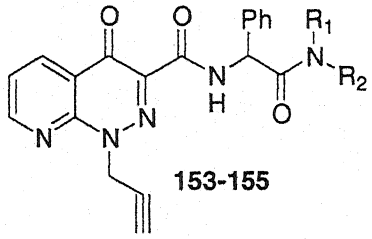


(a) $SOCl_2$; (b) N_2CHCO_2Et; (c) PPh_3, i-Pr_2O; (d) MeOH, H_2O; (e) Propargyl bromide, NaH; (f) aq NaOH; (g) $SOCl_2$;

Table-13: 1-propargyl pyrido[2,3-c]pyridazine-3-carboxamide derivatives (**149-155**)



149-152



153-155

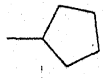
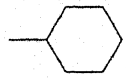
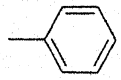
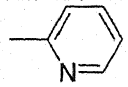
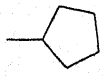
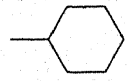
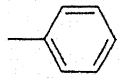
Compound No.	R	Compound No.	R
149		**150**	
151		**152**	
153		**154**	
155			

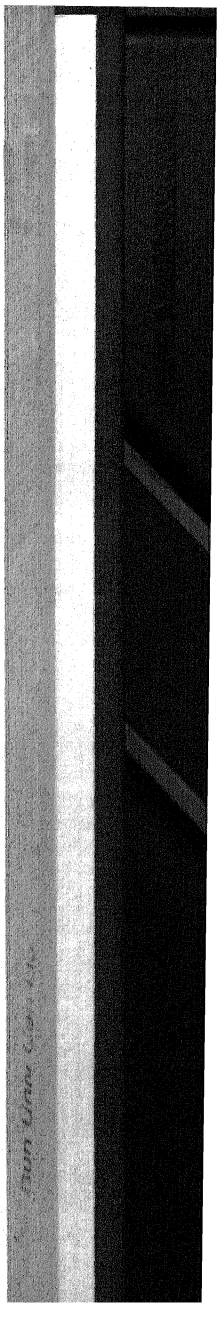
Table 14: *In vitro* cytotoxicity of pyrido[2,3-*c*]pyridazine derivatives (**149-155**)

Compd. No	IC_{50} (μM)									
	PA-1 (Ovary)	DU-145 (Prostate)	KB (Oral)	SW620 (Colon)	HBL100 (Breast)	A549 (Lung)	Miapaca (Pancreas)	K-562 (Leukemia)	NIH3T3 (Normal fibroblast)	CHO (Normal ovary)
152	>10	>10	>10	>10	>10	6.0	8.9	>10	NA	NA
154	>10	>10	9.0	>10	>10	>10	>10	>10	NA	NA
155	>10	>10	8.3	>10	>10	>10	>10	>10	NA	NA

NA = 'Not active' on more than 10 μM concentration

CHAPTER-5

EXPERIMENTAL SECTION



5. EXPERIMENTAL SECTION

All the solvents and reagents were purchased from different companies such as Aldrich, Lancaster, Across & Rankem and were used as supplied. All TLC data (R_f values) were determined with aluminum sheets coated with silica gel 60 F_{254} (Merck). Visualization was achieved with UV light and iodine vapor. Column chromatography was performed using silica gel (100-200 mesh). Proton Magnetic Resonance (PMR) spectra were recorded on a Bruker 300 MHz instrument using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Micromass Quattro micro™ instrument. The purity of the synthesized compounds was determined on Shimadzu HPLC LC-2010 C HT instrument using gradient system and all the compounds showed purity ~99%. Melting points were obtained in a capillary tube with a thermal scientific melting point apparatus Mettler Toledo and are uncorrected.

5.1. Synthesis of betulinic acid derivatives and their respective analytical profile:

5.1.1 3-Oxo-betulinic acid (betulonic acid) (Series 1, Scheme 1) Compound 2:

To a solution of betulinic acid (**1**) (2 g, 4.37 mmol) in acetone (200 ml), Jones reagent (4 ml) was added drop wise (Jone's reagent was prepared by adding 2 mL of H_2SO_4 and 7mL of H_2O to 1.73 gm of CrO_3). The mixture was stirred for 6 h. The resulting solution was filtered, concentrated to dryness under reduced pressure, and extracted with DCM (50 ml). The organic layer was collected, dried over Na_2SO_4, filtered and concentrated to dryness under reduced pressure to provide the 1.6 g (80.4%) of the betulonic acid (**2**).

R_f 0.58 (2% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ: 0.92 (m, 3H), 0.97-1.01 (m, 6H), 1.04 (m, 3H), 1.07 (m, 4H), 1.23-1.25 (m, 2H), 1.33-1.49 (m, 13H), 1.63-1.69 (m, 7H), 1.97-1.99 (m, 3H), 2.17-2.26 (m, 2H), 2.44-2.50 (m, 2H), 4.61 (s, 1H), 4.74-4.76 (s, 1H); MS (ES+) m/z (% relative intensity) 455 (100); HPLC Purity = 100%.

5.1.2 2,3-Didehydroindolo[2',3':2,3]betulinic acid (Series 1, Scheme 1)

Compound 3: To a solution of phenyl hydrazine (0.26g, 2.41 mmol) in ethanol (10ml), 3N HCl (1 ml) was added dropwise (pH~3). To above reaction mixture

solution of betulonic acid (**2**) (1g, 2.19 mmol) in ethanol (10 ml) was added dropwise under stirring. The reaction mixture was refluxed for 6-7h and left overnight. Solvent was then removed in vacuum and residue was extracted with water and DCM. The organic layer was separated, dried over Na_2SO_4 and then evaporated to afford the crude product. The crude product was purified by column using 2%MeOH/DCM as. eluent.

R_f 0.72 (2% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.86 (s, 3H), 1.03 (s, 6H), 1.16 (s, 3H), 1.27 (s, 3H), 1.38-1.82 (m, 20H), 1.98-2.28 (m, 4H), 2.83 (d, 1H, J = 14.9 Hz), 2.9- 3.1 (m, 1H), 4.64 (bs, 1H), 4.7 (bs, 1H), 7.02-7.12 (m, 2H), 7.25-7.39 (m, 2H), 7.69 (bs, 1H); MS (ES+) m/z (% relative intensity) 528 (100); HPLC purity 97.48 %.

5.1.3 5'-Chloro-2,3-didehydroindolo[2',3':2,3]betulinic acid

(Series 1, Scheme 1) Compound 4:

Compound **4** was prepared from coupling of betulonic acid (**2**) with 4-chloro phenylhydrazine, similar to procedure of compound **3**.

R_f 0.62 (2% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.85 (s, 3H), 1.03 (s, 6H), 1.18 (s, 3H), 1.26-1.77 (m, 22H), 1.99-2.2 (m, 3H), 2.27-2.32 (m, 2H), 2.80 (d, 1H, J = 15.0 Hz), 3.0-3.14 (m, 1H), 4.65 (bs, 1H), 4.78 (bs, 1H), 7.03-7.04 (m, 1H), 7.06-7.07 (m, 1H), 7.36 (s, 1H), 7.73 (bs, 1H); MS (ES+) m/z (% relative intensity) 560 (100); HPLC purity 99.09 %.

5.1.4 2,3-Didehydro-5'-methoxyindolo[2',3':2,3]betulinic acid

(Series 1, Scheme 1) Compound 5:

Compound **5** was prepared from coupling of betulonic acid (**2**) with 4-methoxy phenylhydrazine, similar to procedure of compound **3**.

R_f 0.3 (DCM); ^{1}HNMR ($CDCl_3$) δ 0.80 (s, 3H), 0.96 (s, 6H), 1.09 (s, 3H), 1.18-1.21 (m, 9H), 1.25-1.80 (m, 13H), 1.85-2.09 (m, 3H), 2.15-2.30 (m, 2H), 2.72 (d, 1H, J = 14.9 Hz), 2.9-3.1 (m, 1H), 3.76 (s, 3H), 4.56 (bs, 1H), 4.70 (bs, 1H), 6.66-6.70 (m, 1H), 6.78 (d, 1H, J = 2.3 Hz), 7.09 (d, 1H, J = 8.6 Hz), 7.50 (bs, 1H); MS (ES+) m/z (% relative intensity) 556 (100); HPLC purity 99.45 %.

5.1.5 28-*O*-Allyl-2,3-didehydroindolo[2',3':2,3]betulinate

(Series 1, Scheme 1) Compound 6:

2,3-Didehydroindolo[2',3':2,3]betulinic acid (**3**) (2g, 3.78 mmol) and K_2CO_3 (0.78 g, 5.68 mmol) in solvent acetone (50 ml) was stirred for 30 minutes. Then, allyl bromide (1.14g, 2.5 mmol) was added, and the mixture was stirred at rt overnight. Additional allyl bromide (0.68 g, 5.67 mmol) was again added and stirred for 24 hours at room temperature. The solvent was evaporated in vacuum and the residue, thus obtained, was washed with water and hexane. It was then extracted with DCM. The organic layer was combined, dried over Na_2SO_4 and then evaporated to furnish a crude product. The product was purified by column using 20% EtOAc/Hexane as eluent.

R_f 0.4 (20% EtOAc/Hexane); ^{1}HNMR ($CDCl_3$) δ 0.86 (s, 3H), 1.01-1.75 (m, 31H), 1.8-2.0 (m, 2H), 2.05-2.45 (m, 3H), 2.83 (d, 1H, J = 14.9 Hz), 3.05-3.15 (m, 1H), 4.58-4.63 (m, 3H), 4.77 (bs, 1H), 5.9-6.0 (m, 1H), 7.04-7.10 (m, 2H), 7.28-7.3 (m, 1H), 7.37-7.38 (m, 1H), 7.71 (bs, 1H); MS (ES+) m/z (% relative intensity) 566 (95), 113 (100); HPLC purity 100%.

5.1.6 28-*O*-Pivaloyloxymethyl-2,3-didehydroindolo[2',3':2,3]betulinate

(Series 1, Scheme 1) Compound 7:

Compound **7** was prepared from esterification of 2,3-didehydroindolo[2',3':2,3]betulinic acid (**3**) with pivaloyloxymethyl chloride, similar to procedure of compound **6**.

R_f 0.87 (2% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.90 (s, 3H), 1.06 (s, 6H), 1.22-1.75 (m, 34H), 1.8-2.0 (m, 2H), 2.1-2.4 (m, 3H), 2.86 (d, 1H, J = 14.9 Hz), 3.05-3.08 (m, 1H), 4.68 (bs, 1H), 4.81 (bs, 1H), 5.83 (dd, 2H, J = 5.4, 11.0 Hz), 7.06-7.16 (m, 2H), 7.30 (d, 1H, J = 7.3 Hz), 7.42 (d, 1H, J = 7.0 Hz), 7.90 (bs, 1H); MS (ES+) m/z (% relative intensity) 664 (100), 642 (37); HPLC purity 92.19 %.

5.1.7 28-*O*-Benzyl-2,3-didehydro[2',3':2,3]indolobetulinate

(Series 1, Scheme 1) Compound 8:

Compound **8** was prepared from esterification of 2,3-didehydroindolo[2',3':2,3]betulinic acid (**3**) with benzyl bromide, similar to procedure of compound **6**.

R_f 0.9 (2% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.87 (s, 3H), 1.02 (s, 6H), 1.20 (s, 3H), 1.29-1.98 (m, 24H), 2.0-2.4 (m, 3H), 2.84 (d, 1H, *J* = 14.8 Hz), 3.07-3.10 (m, 1H), 4.64 (bs, 1H), 4.78 (bs, 1H), 5.15 (dd, 2H, *J* = 12.3, 20.4 Hz), 7.06-7.14 (m, 3H), 7.28-7.40 (m, 6H), 7.73 (bs, 1H); MS (ES+) m/z (% relative intensity) 618 (100); HPLC purity 94.56 %.

5.1.8 28-*O*-(*p*-nitro)Benzyl-2,3-didehydro[2',3':2,3]indolobetulinate (9)

(Series 1, Scheme 1) Compound 9:

Compound **9** was prepared from esterification of 2,3-didehydroindolo[2',3':2,3]betulinic acid (**3**) with *p*-nitrobenzyl choride, similar to procedure of compound **6**.

R_f 0.6 (DCM); ^{1}HNMR ($CDCl_3$) δ 0.84-2.28 (m, 39H), 2.79-2.84 (m, 1H), 3.02-3.04 (m, 1H), 4.76 (s, 1H), 5.15-5.27 (m, 2H), 7.02-7.71 (m, 7H), 8.25 (d, 1H, J = 8.5); MS (ES+) m/z (% relative intensity) 663 (100); HPLC purity 99.3 %.

5.1.9 28-*O*-Benzyl-5'-chloro-2,3-didehydroindolo[2',3':2,3]betulinate

(Series 1, Scheme 1) Compound 10:

Compound **10** was prepared from esterification of 5'-Chloro-2,3-didehydroindolo[2',3':2,3]betulinic acid (**4**) with benzyl bromide, similar to procedure of compound **6**.

R_f 0.5 (20% EtOAc/Hexane); ^{1}HNMR ($CDCl_3$) δ 0.75-0.76 (m, 6H), 0.92 (s, 3H), 1.0-1.75 (m, 25H), 1.85-1.9 (m, 2H), 1.95-2.30 (m, 3H), 2.68 (d, 1H, *J* = 15.0 Hz), 2.90-3.10 (m, 1H), 4.55 (bs, 1H), 4.68 (bs, 1H), 5.06 (dd, 2H, *J* = 12.3, 20.9 Hz), 6.94-6.98 (m, 1H), 7.08-7.11 (m, 1H), 7.25-7.30 (m, 6H), 7.67 (bs, 1H); MS (ES+) m/z (% relative intensity) 650 (100); HPLC purity 100%.

5.1.10 2,3-Didehydroindolo[2',3':2,3]betulinic acyl chloride (Series 1, Scheme1)

Compound 11:

2,3-Didehydroindolo[2',3':2,3]betulinic acid (**3**) (1 g, 1.89 mmol) was dissolved in DCM (20 ml) and oxalyl chloride (0.36 g, 2.84 mmol) was added and stirred for 6 h at room temperature. It was then evaporated; washed with water, treated with aqueous $NaHCO_3$ solution to pH ~ 6 and extracted with DCM. The combined organic layer

was dried over Na_2SO_4 and then evaporated *in vacuuo* to afford compound **11**, which was used for next step without purification.

5.1.11 28-*N*-Benzyl-2,3-didehydro-20,29-dihydro[2',3':2,3]indolobetulinamide (Series 1, Scheme 1) Compound 12:

Benzyl amine (0.338 g, 3.15 mmol) was added to the solution of compound **11** in DCM (20 ml) and stirred over night at room temperature. The solvent was then evaporated in vacuum and the residue, thus obtained, was washed with water and hexane. It was then extracted with DCM. The organic layer was combined, dried over Na_2SO_4 and then evaporated to furnish crude product. The product was purified by column using 0.5% MeOH/ DCM as eluent.

R_f 0.6 (DCM); ^{1}HNMR ($CDCl_3$) δ 2.15 (m, 41H), 2.17-2.19 (m, 1H), 2.80-2.85 (m, 1H), 3.10-3.12 (m, 1H), 4.39-4.40 (m, 1H), 4.78 (s, 1H), 5.89 (s, 1H), 7.09-7.69 (m, 8H), 7.72 (s, 1H); MS (ES+) m/z (% relative intensity) 616 (100); purity 98.5%. .

5.1.12 3-*O*-acetyl betulinic acid (13) (Series 1, Scheme 2) Compound 13:

Betulinic acid (1g, 2.18 mmol) was dissolved using pyridine (5 ml) in presence of acetic anhydride (1.08 g, 10.66 mmol). The reaction mixture was heated for 5 hr at 50-52°C and poured into ice-cold water. The solid thus separated was filtered, suspended in 100 mL of distilled water, stirred for 2-hrs, filtered, washed with water (50 ml) and dried in oven at 100°C for 24 hr to afford the crude compound, which was further purified by column chromatography.

R_f = 0.5 (2% Methanol/ DCM); ^{1}H NMR ($CDCl_3$) δ: 0.74-0.76 (m, 3H), 0.83-0.84 (m,12H), 0.85-0.94 (m, 6H), 1.30-1.38 (m, 10H), 1.48-1.49 (m, 6H), 1.62 (m, 6H), 1.65 (m, 2H), 2.04 (s, 3H), 2.20-2.22 (m, 3H), 4.45-4.50 (m, 1H), 4.61 (bs, 1H), 4.7 (bs, 1H); MS (ES+) m/z (% relative intensity) 497 (100); HPLC purity 95.0%.

5.1.13 3-*O*-acetyl dihydrobetulinic acid (Series 1, Scheme 2) Compound 14:

3-*O*-acetyl betulinic acid (**13**) was dissolved in ethyl acetate and hydrogenation was carried out using 10% Pd/C in hydrogen environment at 75 psi for 48h. The resulting solution was filtered out and filtrate was concentrated to give the desired product. The crude product was purified by column chromatography using 2% methanol in dichloromethane as mobile phase.

R_f 0.51 (2% Methanol/ DCM) ^{1}H NMR ($CDCl_3$) δ: 0.74-0.76 (m, 3H), 0.83-0.84 (m, 12H), 0.85-0.94 (m, 6H), 1.30-1.38 (m, 10H), 1.48-1.49 (m, 6H), 1.62 (m, 6H), 1.65 (m, 2H), 2.04 (s, 3H), 2.20-2.22 (m, 3H), 4.45-4.50 (m, 1H); MS (ES+) m/z (% relative intensity) 499 (100) HPLC purity 98%.

5.1.14 Dihydrobetulonic acid: (Series 1, Scheme 2) Compound 15:

To a solution of 3-*O*-acetyl dihydrobetulinic acid (**14**) (6 g, 11.98 mmol) in methanol (10 ml), and (15 ml) methanolic NaOH solution (5 g of NaOH in 300 ml MeOH) was added drop wise. The stirring was continued for 4 hrs. Solvent was evaporated under vacuum and further stirred in distilled water for 15 min. Reaction mixture was then neutralized by adding conc. HCl. Solid product was filtered and dried. The crude product was purified by column chromatography using 2% MeOH/DCM.

R_f 0.4 (2% Methanol/ DCM); ^{1}H NMR ($CDCl_3$) δ: 0.74-0.93 (m, 21H), 1.12-1.57 (m, 21H), 1.79-1.81 (m, 2H), 2.24-2.35 (m, 2H), 2.58 (m, 1H), 3.14 (m, 1H); MS (ES+) m/z (% relative intensity) 457 (100); HPLC purity 84.4%.

5.1.15 Dihydrobetulonic acid (16): (Series 1, Scheme 2) Compound 16:

Compound **16** was prepared from oxidation of dihydrobetulinic acid (**15**) similar to procedure of compound **2**.

R_f 0.9 (2% Methanol/ DCM); ^{1}H NMR ($CDCl_3$) δ: 0.92 (s, 3H), 0.97-0.99 (m, 6H), 1.02 (s, 3H), 1.07 (s, 3H), 1.23-1.67 (m, 21H), 1.7-2.0 (m, 3H), 2.17-2.26 (m, 3H), 2.45-2.51 (m, 2H), 3.00-3.02 (m, 1H), 4.61 (s, 1H), 4.74 (s, 1H); MS (ES-) m/z (% relative intensity) 453 (100); HPLC purity 100%.

5.1.16 2,3-Didehydro-20,29-dihydroindolo[2',3':2,3]betulinic acid (Series 1, Scheme 2) Compound 17:

Compound **17** was prepared from coupling of dihydrobetulonic acid (**16**) with phenylhydrazine, similar to procedure of compound **3**.

R_f 0.59 (2% MeOH/$CHCl_3$); ^{1}HNMR ($CDCl_3$) δ 0.78 (d, 3H, J = 6.7 Hz), 0.85-1.02 (m, 12H), 1.15 (s, 3H), 1.24-1.9 (m, 23H), 1.98-2.3 (m, 3H), 2.84 (d, 1H, J = 14.9 Hz), 7.03-7.13 (m, 2H), 7.24-7.29 (m, 1H), 7.40-7.42 (m, 1H), 7.68 (bs, 1H); MS m/z (% relative intensity) 529 (100); HPLC purity 96.87 %.

5.1.17 28-*O*-Benzyl-2,3-didehydro-20,29-dihydro[2',3':2,3]indolobetulinate (18) (Series 1, Scheme 2) Compound 18:

Compound **18** was prepared from esterification of 2,3-didehydroindolo-20,29-dihydroindolo[2',3':2,3]betulinic acid (**17**) with benzyl bromide, similar to procedure of compound **6**.

R_f 0.8 (DCM); ^{1}HNMR ($CDCl_3$) δ 0.79 (d, 3H, J = 6.7 Hz), 0.85-0.91 (m, 12H), 1.0 (s, 3H), 1.20-2.02 (m, 23H), 2.30-2.48 (m, 3H), 2.85 (d, 1H, *J* = 14.9 Hz), 5.04 (dd, 2H, *J* = 12.3, 17.4 Hz), 6.97-7.05 (m, 3H), 7.19-7.34 (m, 6H), 7.68 (bs, 1H); MS (ES+) m/z (% relative intensity) 620 (100); HPLC purity 97.8 %.

5.2. Synthesis of functionalized amino acid derivatives and their respective analytical profile:

5.2.1. Procedure for the synthesis of Functionalized amino acid derivatives (Series 2, Scheme 3) (Compound 20-53):

5.2.1.1 5-*N*-Isopropyl-3-*N'*-*tert*-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 20):

Isopropyl amine (0.91 g, 15.56 mmol) was added, to a stirred solution of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid (5 g, 15.56 mmol) in dichloromethane (100 ml). The resulting solution was placed in ice bath for 15 min. at 0°C. To this reaction mixture 1-hydroxybezotriazole hydrate (HOBt, 2.10 g, 15.56 mmol) and *N*-methylmorpholine (NMM, 1.57 g, 15.56 mmol) were added. After stirring for 30 min. at 0°C, N-ethyl-N'-3-dimethylaminopropyl carbodiimide hydrochloride (EDCI, 3.20 g, 15.56 mmol) was added and the reaction mixture was maintained at 0°C for 3h, then stirred for 5h at rt and left overnight. Water (100 ml) was added to reaction mixture and extracted with dichloromethane (100 ml). The combined organic layer was dried over Na_2SO_4 and evaporated to afford the crude residue. The crude product was purified by column chromatography using 3% methanol/dichloromethane as eluent, to provide the pure compound **20**.

R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.16-1.30 (m, 15H), 1.70 (s, 3H), 1.78 (s, 3H), 4.06-4.17 (m, 1H), 4.33 (d, 1H, J = 5.6 Hz), 5.09 (bs, 1H), 6.33 (bs, 1H), 7.24-7.34 (m, 5H); MS (ES+) m/z (relative intensity) 363 (M+H) (10), 385 (M+H+Na) (100); HPLC purity = 95.7 %.

5.2.1.2 5-*N*-Cyclopropyl-3-*N'*-*tert*-butoxycarbonyl-2,2-dimethyl-4- phenyl oxazolidine-5- carboxamide (Compound 21):

Compound **21** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with cyclopropyl amine, similar to procedure of compound **20**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.54-0.56 (m, 2H), 0.80-0.82 (m, 2H), 1.14 (bs, 9H), 1.68 (s, 3H), 1.75 (s, 3H), 2.73-2.76 (m, 1H), 4.32 (d, 1H, J = 5.8 Hz), 5.06 (bs, 1H), 6.57 (bs, 1H), 7.26-7.42 (m, 5H); MS (ES+) m/z (relative intensity) 383 (M+H+Na) (100); HPLC purity = 99.7 %.

5.2.1.3 5-*N*-Cyclopentyl-3-*N'*-*tert*-butoxycarbonyl-2,2-dimethyl-4- phenyl oxazolidine-5- carboxamide (Compound 22):

Compound **22** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with cyclopentyl amine, similar to procedure of compound **20**.

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.15 (bs, 9H), 1.38-1.46 (m, 1H), 1.63-1.69 (m, 8H), 1.77 (s, 3H), 1.96-2.0 (m, 2H), 4.18 – 4.28 (m, 1H), 4.33 (d, 1H, J = 5.5 Hz), 5.11 (bs, 1H), 6.43 (d, 1H, J = 6.9 Hz), 7.34-7.24 (m, 5H); MS (ES+) m/z (relative intensity) 389 (M+H) (10), 411 (M+H+Na) (100); HPLC purity = 99.5 %.

5.2.1.4 5-*N*-Cyclohexyl-3-*N'*-*tert*-butoxycarbonyl-2,2-dimethyl-4- phenyl oxazolidine-5- carboxamide (Compound 23):

Compound **23** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with cyclohexyl amine, similar to procedure of compound **20**.

R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.04-1.27 (m, 11H), 1.31-1.44 (m, 3H), 1.60-1.82 (m, 9H), 1.88-1.95 (m, 2H), 3.78-3.81 (m, 1H), 4.32 (d, 1H, J = 5.5 Hz), 5.09 (bs, 1H), 6.41 (d, 1H, J = 7.9 Hz), 7.22-7.36 (m, 5H); MS (ES+) m/z (relative intensity) 403 (M+H) (30), 425 (M+H+Na) (100); HPLC purity = 99.3 %.

5.2.1.5 5-*N*-Phenyl-3-*N'*-*tert*-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 24):

Compound **24** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with aniline, similar to procedure of compound **20**.

R_f 0.4 (20% EtOAc/Hexane); ^{1}HNMR ($CDCl_3$) δ 1.08 (bs, 9H), 1.72 (s, 3H), 1.76 (s, 3H), 4.44 (d, 1H, J = 5.6 Hz), 5.12 (bs, 1H), 7.07 (t, 1H, J = 7.2 Hz), 7.18-7.30 (m, 7H), 7.49-7.52 (m, 2H), 8.22 (bs, 1H); MS (ES+) m/z (relative intensity) 419 (M+H+Na) (100); HPLC purity = 93.8 %.

5.2.1.6 5-*N*-(4'-Fluoro) phenyl-3-*N'*-*tert*-butoxycarbonyl-2,2-dimethyl-4- phenyl oxazolidine-5- carboxamide (Compound 25):

Compound **25** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 4-fluoro aniline, similar to procedure of compound **20**.

R_f 0.3 (DCM); ^{1}HNMR ($CDCl_3$) δ 1.16 (bs, 9H), 1.78 (s, 3H), 1.82 (s, 3H), 4.51 (d, 1H, *J* = 5.9 Hz), 5.16 (bs, 1H), 7.01-7.06 (m, 2H), 7.26-7.37 (m, 5H), 7.51-7.56 (m, 2H), 8.28 (bs, 1H); MS (ES+) m/z (relative intensity) 437 (M+H+Na) (100).

5.2.1.7 5-*N*-(4'-cyano) phenyl -3-*N'*- *tert*-butoxycarbonyl - 2,2 - dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 26):

Compound **26** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 4-cyano aniline, similar to procedure of compound **20**.

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.14-1.17 (m, 9H), 1.78-1.83 (m, 6H), 4.52 (d, 1H, *J* = 6.0 Hz), 5.15 (bs, 1H), 7.25-7.37 (m , 5H), 7.63-7.73 (m, 5H, 8.45 (bs, 1H); MS (ES-) m/z (relative intensity) 420 (M-H) (100); HPLC purity = 98.3 %.

5.2.1.8 5-*N*-(4'-Methoxy) phenyl -3-*N'*- *tert*-butoxycarbonyl - 2,2 - dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 27):

Compound **27** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 4-methoxy aniline, similar to procedure of compound **20**.

R_f 0.2 (DCM); ^{1}HNMR ($CDCl_3$) δ 1.09 (bs, 9H), 1.71 (s, 3H), 1.75 (s, 3H), 3.78 (s, 3H), 4.43 (d, 1H, *J* = 5.7 Hz), 5.11 (bs, 1H), 6.79-6.82 (m, 2H), 7.18-7.32 (m, 5H), 7.39-7.42 (m, 2H), 8.11 (bs, 1H); MS (ES+) m/z (relative intensity) 427 (M+H) (10), 449 (M+H+Na) (100); HPLC purity = 95.9 %.

5.2.1.9 5-*N*-(3'-Chloro-4'-fluoro) phenyl -3-*N'*- *tert*-butoxycarbonyl - 2,2 - dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 28):

Compound **28** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 3-chloro-4-fluoro aniline, similar to procedure of compound **20**.

R_f 0.5 (DCM); ^{1}HNMR ($CDCl_3$) δ 1.09 (bs, 9H), 1.70 (s, 3H), 1.74 (s, 3H), 4.42 (d, 1H, *J* = 5.9 Hz), 5.19 (bs, 1H), 7.03 (t, 1H, *J* = 8.7 Hz), 7.18-7.30 (m, 6H), 7.70-7.72 (m, 1H), 8.20 (bs, 1H) ; MS (ES+) m/z (relative intensity) 449 (M+H) (5), 471 (M+H+Na) (100); HPLC purity = 96.4 %.

5.2.1.10 5-*N*-Benzyl-3-*N*'- *tert*-butoxycarbonyl - 2,2 - dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 29):

Compound **29** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with benzylamine, similar to procedure of compound **20**.

R_f 0.5 (15% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.14 (bs, 9H), 1.66 (s, 3H), 1.75 (s, 3H), 4.41-4.58 (m, 3H), 5.09 (bs, 1H), 6.84 (bs, 1H), 7.24-7.35 (m, 10H); MS (ES+) m/z (relative intensity) 433 (M+H+Na) (100); HPLC purity = 99.7 %.

5.2.1.11 5-*N*-2'-Pyridine- 3- *N*'- *tert*- butoxycarbonyl- 2, 2- dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 30):

Compound **30** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 2-amino pyridine, similar to procedure of compound **20**.

R_f 0.6 (2% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.14 (bs, 9H), 1.79 (s, 3H), 1.82 (s, 3H), 4.51 (d, 1H, *J* = 5.9 Hz), 5.16 (bs, 1H), 7.06-7.1 (m, 1H), 7.26-7.46 (m, 5H), 7.7-7.75 (m, 1H), 8.24 (d, 1H, *J* = 8.2 Hz), 8.31-8.32 (m, 1H), 8.91 (bs, 1H); MS (ES+) m/z (relative intensity) 398 (M+H) (10), 420 (M+H+Na) (100); HPLC purity = 95.5 %.

5.2.1.12 5-*N*-3'-Pyridine- 3- *N*'- *tert*- butoxycarbonyl- 2, 2- dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 31):

Compound **31** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 3-amino pyridine, similar to procedure of compound **20**.

R_f 0.7 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.16 (bs, 9H), 1.70 (s, 3H), 1.76 (s, 3H), 4.53 (d, 1H, *J* = 6.0 Hz), 5.17 (bs, 1H), 7.26-7.46 (m, 6H), 8.23 (d, 1H, *J* = 8.0

Hz), 8.36-8.4 (m, 2H), 8.64 (bs, 1H); MS (ES+) m/z (relative intensity) 398 (M+H) (30), 420 (M+H+Na) (100); HPLC purity = 95.8 %.

5.2.1.13 5-*N*-4'-Pyridine- 3- *N'*- *tert*- butoxycarbonyl- 2, 2- dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 32):

4-Amino pyridine (1.46 g, 15.56 mmol) and 4-dimethylaminopyridine (DMAP, 1.90 g, 15.56 mmol) were added to a stirred solution of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid (5 g, 15.56 mmol) in dichloromethane (100 ml). The reaction mixture was placed in ice bath and after 30 min at 0°C, to this *N,N'*-Dicyclohexyl-Carbodiimide (DCC, 3.21 g, 15.56 mmol) was added under nitrogen condition. The reaction mixture was further stirred for 5h at rt and left overnight. Water (100 ml) was added to reaction mixture and extracted with dichloromethane (100 ml). The combined organic layer was dried over Na_2SO_4 and evaporated to afford the crude residue. The crude product was purified by column chromatography using 2% methanol/ dichloromethane as eluent. In several cases, solid was appeared during the addition of water in the reaction mixture. It was filtered, washed with water, dried and purified, as described above, to provide the pure compound.

R_f 0.7 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.17 (bs, 9H), 1.78 (s, 3H), 1.83 (s, 3H), 4.51 (d, 1H, *J* = 6.0 Hz), 5.16 (bs, 1H), 7.26-7.38 (m, 5H), 7.52-7.54 (m, 2H), 8.41 (bs, 1H), 8.53-8.55 (m, 2H); MS (ES+) m/z (relative intensity) 398 (M+H) (100), 420 (M+H+Na) (10); HPLC purity = 92.8 %.

5.2.1.14 5-*N*-2'-Thiazole- 3- *N'*- *tert*- butoxycarbonyl- 2, 2- dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 33):

Compound **33** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 2-amino thiazole, similar to procedure of compound **20**.

R_f 0.6 (2% MeOH/DCM); ^{1}HNMR ,($CDCl_3$) δ 1.16 (bs, 9H), 1.75 (s, 3H), 1.81 (s, 3H), 4.60 (d, 1H, *J* = 5.9 Hz), 5.19 (bs, 1H), 7.03 (d, 1H, *J* = 3.5 Hz), 7.27-7.38 (m, 5H), 7.49 (d, 1H, *J* = 3.5 Hz), 9.92 (bs, 1H); MS (ES+) m/z (relative intensity) 404 (M+H) (5), 426 (M+H+Na) (100); HPLC purity = 98.3 %.

5.2.1.15 5-*N*-Piperidine- 3- *N'*- *tert*- butoxycarbonyl- 2, 2- dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 34):

Compound **34** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with piperidine, similar to procedure of compound **20**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.13 (bs, 9H), 1.54-1.63 (m, 9H), 1.76 (s, 3H), 3.25-3.38 (m, 2H), 3.48-3.54 (m, 1H), 3.74-3.78 (m, 1H), 4.54 (d, 1H, J = 5.0 Hz), 5.53 (bs, 1H), 7.24-7.36 (m, 5H); MS (ES+) m/z (relative intensity) 389 (M+H) (10), 411 (M+H+Na) (100); HPLC purity = 99 %.

5.2.1.16 5-*N*-Morpholine- 3- *N'*- *tert*- butoxycarbonyl- 2, 2- dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 35):

Compound **35** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with morpholine, similar to procedure of compound **20**.

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.07-1.37 (m, 9H), 1.51 (s, 3H), 1.67 (s, 3H), 3.39-3.52 (m, 8H), 4.76 (d, 1H, J = 4.6 Hz), 5.32 (bs, 1H), 7.26-7.37 (m, 5H); MS (ES+) m/z (relative intensity) 391 (M+H) (5), 413 (M+H+Na) (100); HPLC purity = 99.6 %.

5.2.1.17 5-*N*-Pyrrolodine- 3- *N'*- *tert*- butoxycarbonyl- 2, 2- dimethyl-4-phenyl oxazolidine-5- carboxamide (Compound 36):

Compound **36** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with pyrrolidine, similar to procedure of compound **32**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.11 (bs, 9H), 1.62 (s, 3H), 1.66 (s, 3H), 1.76-1.91 (m, 4H), 3.19-3.21 (m, 1H), 3.46-3.5 (m, 2H), 3.62-3.64 (m, 1H), 4.44 (d, 1H, J = 6.0 Hz), 5.41 (bs, 1H), 7.24-7.33 (m, 5H); MS (ES+) m/z (relative intensity) 397 (M+H+Na) (100), HPLC purity = 94.6 %.

5.2.1.18 1-*N*-Isopropyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 37):

To 5-*N*-Isopropyl-3-*N'*-*tert*-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide (**20**, 5 g, 13.79 mmol), 50 % TFA/DCM (50 ml) was added at 0 ^{0}C. Reaction mixture was stirred for 4h at rt ant then left overnight. Aqueous $NaHCO_3$ saturated solution was then added till the neutralization. DCM layer was separated, dried over Na_2SO_4 and evaporated to afford the crude product. The product was further purified by column chromatography using 2% MeOH/DCM as eluent.

R_f 0.2 (15% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.02-1.18 (m, 6H), 2.20 (bs, 3H), 3.99-4.1 (m, 2H), 4.57 (d, 1H, J = 2.7 Hz), 6.62 (d, 1H, J = 7.3 Hz), 7.18-7.64 (m, 5H); MS (ES+) m/z (relative intensity) 223 (M+H) (100), 245 (M+H+Na) (90), HPLC purity = 80.7 %.

Compounds **38-53** were prepared in the similar way to compound **37**.

5.2.1.19 1-*N*-Cyclopropyl-3-amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 38):

R_f 0.2 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.47-0.50 (m, 2H), 0.75-0.78 (m, 2H), 1.98 (bs, 3H), 2.68-2.75 (m, 1H), 4.03 (d, 1H, J = 2.8 Hz), 4.58 (d, 1H, J = 2.8 Hz), 6.9 (bs, 1H), 7.28-7.45 (m, 5H); MS (ES+) m/z (relative intensity) 221 (M+H) (20), 243 (M+H+Na) (100), HPLC purity = 94.6 %.

5.2.1.20 1-*N*-Cyclopentyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 39):

R_f 0.3 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.25-1.39 (m, 2H), 1.59-1.63 (m, 3H), 1.91-2.0 (m, 3H), 4.03 (d, 1H, J = 2.8 Hz), 4.14-4.23 (m, 1H), 4.57 (d, 1H, J = 2.8 Hz), 6.74 (bs, 1H), 7.26-7.41 (m, 5H); MS (ES+) m/z (relative intensity) 249 (M+H) (30), 271 (M+H+Na) (100), HPLC purity = ~ 100 %.

5.2.1.21 1-*N*-Cyclohexyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 40):

R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.05-1.42 (m, 5H), 1.59-1.84 (m, 5H), 2.32 (bs, 2H), 3.76-3.79 (m, 1H), 4.0 (s, 1H), 4.53 (s, 1H), 6.67 (d, 1H, J = 7.1 Hz),

7.26-7.40 (m, 5H); MS (ES+) m/z (relative intensity) 263 (M+H) (60), 285 (M+H+Na) (100), HPLC purity = 94.7 %.

5.2.1.22 1-*N*-Phenyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 41):

R_f 0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.90 (bs, 2H), 4.08 (s, 1H), 4.20 (s, 1H), 5.8 (bs, 1H), 7.01-7.06 (m, 1H), 7.17-7.19 (m, 1H), 7.20-7.30 (m, 4H), 7.39 (d, 2H, J = 7.4 Hz), 7.64 (d, 2H, J = 7.9 Hz), 9.63 (bs, 1H); MS (ES+) m/z (relative intensity) 257 (M+H) (5), 279 (M+H+Na) (100), HPLC purity = ~ 100 %.

5.2.1.23 1-*N*-(4'-Fluoro) phenyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 42):

R_f 0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.90 (bs, 2H), 4.07 (s, 1H), 4.20 (s, 1H), 5.75 (bs, 1H), 7.09-7.49 (m, 7H), 7.64-7.69 (m, 2H), 9.72 (bs, 1H); MS (ES+) m/z (relative intensity) 275 (M+H) (25), 297 (M+H+Na) (100), HPLC purity = 95 %.

5.2.1.24 1-*N*-(4'-Cyano) phenyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 43):

R_f 0.2 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 4.13 (d, 1H, J = 3.9 Hz), 4.22 (d, 1H, J = 3.9 Hz), 7.18-7.40 (m, 5H), 7.73 (d, 2H, J = 8.6 Hz), 7.85 (d, 2H, J = 8.6 Hz); MS (ES+) m/z (relative intensity) 282 (M+H) (40), 304 (M+H+Na) (70), HPLC purity = 92.3 %.

5.2.1.25 1-*N*-(4'-Methoxy) phenyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 44):

R_f 0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.88 (bs, 2H), 3.71 (s, 3H), 4.04 (d, 1H, J = 3.0 Hz), 4.19 (s, 1H), 5.7 (bs, 1H), 6.86 (d, 2H, J = 8.7 Hz), 7.17-7.40 (m, 5H), 7.52-7.55 (m, 2H), 9.51 (bs, 1H); MS (ES+) m/z (relative intensity) 287 (M+H) (30), 309 (M+H+Na) (100), HPLC purity = 94.2 %.

5.2.1.26 1-*N*-(3'-Chloro-4'-fluoro) phenyl - 3- amino- 2-hydroxy -3-phenylpropane-1-carboxamide (Compound 45):

R_f 0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 4.07 (d, 1H, J = 3.6 Hz), 4.19 (d, 1H, J = 3.6 Hz), 7.17-7.39 (m, 6H), 7.58-7.63 (m, 1H), 7.99 (dd, 1H, J = 2.5, 6.9 Hz); MS (ES+) m/z (relative intensity) 309 (M+H) (40), 331 (M+H+Na) (100), HPLC purity = 90.8 %.

5.2.1.27 1-*N*-Benzyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 46):

R_f 0.5 (15% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 8.23 (bs, 1H), 7.37-7.15 (m, 10H), 5.54 (bs, 1H), 4.27 (d, 2H, J = 6.0 Hz), 4.14 (d, 1H, J = 2.9 Hz), 3.99 (s, 1H), 1.81 (bs, 2H); MS (ES+) m/z (relative intensity) 271 (M+H) (5), 293 (M+H+Na) (100), HPLC purity = 98.7 %.

5.2.1.28 1-*N*-2'-Pyridine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 47):

R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 4.31 (d, 1H, J = 1.7 Hz), 4.71 (s, 1H), 6.88-6.92 (m, 1H), 7.26-7.47 (m, 6H), 7.65-7.7 (m, 1H), 8.27 (d, 1H, J = 8.3 Hz), 9.83 (bs, 1H); MS (ES+) m/z (relative intensity) 258 (M+H) (5), 280 (M+H+Na) (100), HPLC purity = 99.2 %.

5.2.1.29 1-*N*-3'-Pyridine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 48):

R_f 0.2 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 4.26 (s, 1H), 4.72 (s, 1H), 7.28-7.44 (m, 6H), 8.20 (d, 1H, J = 8.4 Hz), 8.35 (d, 1H, J = 4.5 Hz), 8.61 (s, 1H), 9.16 (bs, 1H); MS (ES+) m/z (relative intensity) 258 (M+H) (80), 280 (M+H+Na) (100), HPLC purity = ~ 100 %.

5.2.1.30 1-*N*-4'-Pyridine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 49):

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 4.16 (s, 1H, J = 4.2 Hz), 4.24 (d, 1H, J = 4.2 Hz), 7.19-7.40 (m, 5H), 7.64 (d, 2H, J = 6.2 Hz), 8.39 (d, 2H, J = 6.2 Hz); MS (ES+) m/z (relative intensity) 258 (M+H) (15), 280 (M+H+Na) (35).

5.2.1.31 1-*N*-2'-Thiazole- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 50):

R_f 0.3 (5% MeOH/DCM) ^{1}HNMR ($CDCl_3$) δ 1.25 (s, 2H), 4.37 (d, 1H, *J* = 1.8 Hz), 4.71 (d, 1H, *J* = 1.8 Hz), 6.97 (d, 1H, *J* = 3.5 Hz), 7.31-7.43 (m, 6H); MS (ES+) m/z (relative intensity) 264 (M+H) (100); HPLC purity = 96.8 %.

5.2.1.32 1-*N*-Piperidine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 51):

R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.19-1.25 (m, 1H), 1.38-1.5 (m, 5H), 2.48 (bs, 3H), 2.86-2.90 (m, 1H), 3.20-3.37 (m, 2H), 3.67-3.71 (m, 1H), 4.07 (d, 1H, *J* = 4.5 Hz), 4.44 (d, 1H, *J* = 4.5 Hz), 7.25-7.42 (m, 5H); MS (ES+) m/z (relative intensity) 249 (M+H) (50), 271 (M+H+Na) (100), HPLC purity = 94 %.

5.2.1.33 1-*N*-Morpholine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 52):

R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.29 (bs, 3H), 2.80-2.85 (m, 1H), 2.98-3.03 (m, 1H), 3.20-3.24 (m, 1H), 3.30-3.63 (m, 5H), 4.12 (d, 1H, *J* = 5.9 Hz), 4.35 (d, 1H, *J* = 5.9 Hz), 7.26-7.42 (m, 5H); MS (ES+) m/z (relative intensity) 251 (M+H) (50), 273 (M+H+Na) (100), HPLC purity = 98.8 %.

5.2.1.34 1-*N*-Pyrrolodine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (Compound 53):

R_f 0.2 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.52-1.77 (m, 4H), 2.31 (bs, 3H), 2.52-2.55 (m, 1H), 3.22-3.28 (m, 2H), 3.33-3.37 (m, 1H), 4.12-4.18 (m, 2H), 7.25-7.35 (m, 3H), 7.40 –7.43 (m, 2H); MS (ES+) m/z (relative intensity) 235 (M+H) (50), 257 (M+H+Na) (100), HPLC purity = 97.8 %.

5.2.2. Procedure for the synthesis of Functionalized amino acid derivatives (Series 3 and 4) (Scheme 4 and 5) (60-63 and 69-71):

5.2.2.1 (DL)-3-*tert*-Butoxycarbonylamino-3-phenyl-propionic acid (Compound 55):

Di-*tert*-butyl pyrocarbonate (7.26 g, 33.26 mmol) was added in portions to the stirred solution of (DL)-2-amino phenyl propionic acid (5 g, 30.26 mmol) in dioxane and water (2:1) (50 ml) and 1 N NaOH (10 ml) at 0-5° C. The reaction mixture was stirred at ambient temperature for 30 minute and concentrated in vacuum. The resulting mixture was cooled at 0-5° C, ethyl acetate (20 ml) was added and acidified with dilute hydrochloric acid to pH 2-3. The organic layer was separated and aqueous layer was extracted with ethyl acetate three times (3*25 ml). The ethyl acetate extracts were combined, washed with water, dried over Na_2SO_4 and concentrated in vacuum to furnish the titled compound **55**.

5.2.2.2 (DL)-*N*-*tert*-butoxycarbonylanilino-3-phenyl-propionamides (Compound 58):

Aniline (1.75 g, 18.84 mmol) was added, to a stirred solution of (DL)-3-*tert*-Butoxycarbonylamino-3-phenyl-propionic acid (**55**) (5 g, 18.84 mmol) in dichloromethane (100 ml). The resulting solution was placed in ice bath for 15 min. at 0°C. To this reaction mixture 1-hydroxybezotriazole hydrate (HOBt, 2.54 g, 18.84 mmol) and *N*-methylmorpholine (NMM, 1.90 g, 18.84 mmol) were added. After stirring for 30 min. at 0°C, N-ethyl-N'-3-dimethylaminopropyl carbodiimide hydrochloride (EDCI, 3.86 g, 18.84 mmol) was added and the reaction mixture was maintained at 0°C for 3h, then stirred for 5h at rt and left overnight. Water (100 ml) was added to reaction mixture and extracted with dichloromethane (100 ml). The combined organic layer was dried over Na_2SO_4 and evaporated to afford the crude residue. The crude product was used as such without purification.

Compound **56**, **57** and **59** were also prepared in the similar way to compound **58** and used as such without purification.

5.2.2.3 Synthesis of DL-3-Amino-3,*N*-diphenyl-propionamide (Compound 62):

To (DL)-*N*-*tert*-butoxycarbonylanilino-3-phenyl-propionamides (**58**) (5 g, 14.68 mmol), 50 % TFA/DCM (50 ml) was added at 0^0C. Reaction mixture was stirred for 4h at rt ant then left overnight. Aqueous $NaHCO_3$ saturated solution was then added till the neutralization. DCM layer was separated, dried over Na_2SO_4 and evaporated to afford the crude product. The product was further purified by column chromatography using 2% MeOH/DCM as eluent.

R_f 0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO) δ 2.45-2.56 (m, 2H), 4.26-4.30 (m, 1H), 6.97-7.02 (m, 1H), 7.16-7.30 (m, 5H), 7.38 (d, 1H, *J* = 7.26 Hz), 7.54 (d, 1H, *J* = 7.64 Hz), 10.05 (s, 1H); MS (ES+) m/z (relative intensity) 241 (M+H) (100).

Similarly, compounds **60**, **61** and **63** were also prepared in the similar manner from **56**, **57** and **59**, respectively.

5.2.2.4 3-Amino-N-cyclopentyl-3-phenyl-propionamide (Compound 60):

R_f 0.4 (10% MeOH/DCM); ^{1}HNMR (DMSO) δ 1.22-1.72 (m, 8H), 2.29 (d, 1H, *J* = 6.87 Hz), 3.90-3.95 (m, 1H), 4.17 (t, 1H, *J* = 6.80 Hz), 7.15-7.34 (m, 5H), 7.83 (d, 1H, *J* = 6.99 Hz),); MS (ES+) 233 (M+H).

5.2.2.5 3-Amino-N-cyclohexyl-3-phenyl-propionamide (Compound 61):

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.05-1.42 (m, 5H), 1.59-1.84 (m, 5H), 2.32 (bs, 2H), 2.73-2.75 (m, 2H), 3.76-3.79 (m, 1H), 4.53 (s, 1H), 6.67 (d, 1H, *J* = 7.1 Hz), 7.26-7.40 (m, 5H), ; MS (ES+) 247 (M+H).

5.2.2.6 3-Amino-3-phenyl-N-pyridin-2-yl-propionamide (Compound 63):

R_f 0.2 (10% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 1.66 (bs, 1H), 2.71-2.73 (m, 2H), 4.30 (s, 1H), 7.31-7.44 (m, 3H), 7.45 (m, 2H), 7.70 (t, 1H, *J* = 8.0 Hz), 7.85 (d, 1H, *J* = 4.44 Hz), 8.24 (d, 1H, *J* = 8.25 Hz); MS (ES+) 242 (M+H).

5.2.2.7 (DL)-*N*-*tert*-butoxycarbonylamino-2-phenylglycine (Compound 65):

Compound **65** was prepared in a way similar to the compound **55** except (DL)-2-phenylglycine was used in place of (DL)-3-aminophenylpropionic acid.

5.2.2.8 (DL)-2-*N*-*tert*-butoxycarbonylamino-2-phenyl acetamides (Compound 66-68):

Similarly, these compounds were prepared as described for **58**, from (DL)-*N*-*tert*-butoxycarbonylamino-2-phenylglycine (**65**) in place of **55**. Compounds **66-68** were used in the next step without further characterization.

Compounds **69-71** were prepared from deprotection of Boc-group from (DL)-2-*N*-*tert*-butoxycarbonylamino-2-phenyl acetamides (**66-68**), similar to the procedure of compound **62**.

5.2.2.9 2-Amino-*N*-cyclopentyl-2-phenyl acetamide (Compound 69):

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR (DMSO) δ 1.0-1.76 (m, 8H), 3.69-3.78 (m, 1H), 5.55 (d, 1H, J = 7.96 Hz), 7.26-7.42 (m, 5H), 8.62 (s, 1H).

5.2.2.10 2-Amino-*N*-cyclohexyl-2-phenyl acetamide (Compound 70):

R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO) δ 1.10-1.72 (m, 10H), 2.18 (s, 1H), 4.27 (s, 1H), 7.17-7.36 (m, 5H), 7.86 (d, 1H, J = 7.86 Hz); MS (ES+) m/z (relative intensity) 233 (M+H) (85), 255 (M+H+Na) (100).

5.2.2.11 2-Amino-2,*N*-diphenyl acetamide (Compound 71):

R_f 0.4 (7% MeOH/DCM); ^{1}HNMR (DMSO) δ 7.02 (t, 1H, J = 7.3 Hz), 7.21-7.62 (m, 9H), 9.99 (bs, 1H); MS (ES+) m/z (relative intensity) 225 (M+H) (100).

5.3 Synthesis of 1,8-naphthyridine-3-carboxamide Derivatives:

5.3.1 Procedure for synthesis of 1,8-naphthyridine-3-carboxamide derivatives of Series 5, Scheme6, Scheme 7 and Scheme 8:

5.3.1.1 Ethyl 2-chloro-3-nicotinoylacetate (Compound 73a):

To a solution of 2-chloro nicotinic acid (**72a**) (5g, 31.73 mmol) in THF (50 ml) was added 1,1'-carbonylbis-1*H*-imidazole (CDI) (6.17 g, 38.08 mmol). The resulting mixture was heated at 60 ℃ for h. This crude imidazolide solution was used without purification in next step. To a solution of ethyl hydrogen malonate (4.9 g, 31.73 mmol) in THF (50 ml) was added dropwise 3 M MeMgBr (7.36 g, 63.47 mmol) in Et_2O under ice cooling. After being stirred for 20 min. the imidazole prepared in the above was added. The reaction mixture was stirred for 60°C for 1.5 h. poured into ice water and acidified to pH 5-6 with concentrated HCl then extracted with EtOAc (2*50 ml). The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with $CHCl_3$.

73a: R_f 0.7 (2% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 1.21-1.36 (m, 3H), 4.08-4.15 (m, 4H), 7.26-738 (m, 1H), 7.93-7.98 (m, 1H), 8.43-8.52 (m, 1H); MS (ES+) m/z (relative intensity) 226 (M+H) (100).

Compounds **73b-d** were prepared, similar to the procedure of compound **73a**.

5.3.1.2 Ethyl 2-chloronicotinoylacrylate (Compound 74a):

A mixture composed of **73a** (5 g, 21.96 mmol), triethyl ortho formate (4.88 g, 32.94 mmol) and acetic anhydride (5.88 g, 54.91 mmol) was heated to reflux for 1.5 h at 130°C, during which period the resulting EtOAc was distilled off under atmospheric pressure. After concentration under reduced pressure, the resulting residue was diluted with *i*-Pr_2O (100 ml) and propargyl amine (1.40 g, 25.47 mmol). After the mixture was stirred at room temperature, the resulting precipitates were collected by filtration and washed with *i*-Pr_2O to give crude compound **74a**, which was used as such with out further purification.

Compounds **74b-f** were prepared, similar to the procedure of compound **74a**.

5.3.1.3 Ethyl-1-propargyl-1,8-naphthyridine-3-carboxylate (Compound 75a):

To a solution of compound **74a** (5 g, 17.08 mmol) in EtOAc (50 ml), K_2CO_3 (3.54 g, 25.62 mmol) was added. The reaction mixture was refluxed for 8h. The reaction mixture was then diluted with water and extracted with chloroform. The organic layer was dried over Na_2SO_4 and concentrated to dryness to afford a crude product, which was chromatographed on silica gel using 3%MeOH/ DCM as eluent to provide pure **75a**.

R_f 0.4 (5 % MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.42 (t, 1H, J = 7.05 Hz), 2.56 (s, 1H), 4.42 (q, 2H, J = 7.02 Hz), 5.23 (s, 1H), 7.45 (dd, 1H, J = 4.7 Hz), 8.75-8.80 (2H, m), 8.92 (s, 1H); MS (ES+) m/z (relative intensity) 257 (M+H) (5), 279 (M+H+Na) (100).

Compounds **75b-f** were prepared, similar to the procedure of compound **75a**.

5.3.1.4 1-propargyl-1,8-naphthyridine-3-carboxylic acid (Compound 76a):

Compound **75a** (2 g, 8.7 mmol) in 30% HCl (25 ml) was heated to reflux for 6h. After ice cooling the resulting precipitates were collected by filtration, washed with 0.5 N HCl and EtOH successively and dried to provide the desired carboxylic acids **76a**.

R_f 0.7 (5 % MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.63 (s, 1H), 5.37 (d, 1H, J = 1.95 Hz), 7.59 (dd, 1H, J = 4.5 Hz), 9.83-8.93 (m, 2H), 9.25 (s, 1H), 14.26 (s, 1H); MS (ES+) m/z (relative intensity) 229 (M+H) (100).

Compounds **76b-f** were prepared, similar to the procedure of compound **76a**.

5.3.1.5 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2- Isopropylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 77):

To a solution of 1-propargyl-1,8-naphthyridine-3-carboxylic acid (1 g, 4.38 mmol) **76a** in dichloromethane (50 ml), 2 drops of DMF and thionyl chloride ((1.46 g, 6.57 mmol)) was added dropwise at room temperature. The stirring was continued for 4h at room temperature and dried under vacuum to provide acyl chloride intermediate. The acyl chloride intermediate was diluted with dichloromethane (20 ml) and 1-*N*-Isopropyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**37**) (0.78 g, 6.57 mmol) was added to it and stirred for 2h. The reaction mixture was left overnight; water (50 ml) was added and extracted with dichloromethane (50 ml). The organic

layer was dried (Na_2SO_4) and concentrated to dryness to provide crude product. The obtained crude product was purified over silica column using 2%MeOH/ DCM as eluent.

R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$ + DMSO-d_6) δ 0.92 (d, 3H, J = 6.5 Hz), 1.09 (d, 3H, J = 6.5 Hz), 2.73-2.74 (m, 1H), 3.96-3.99 (m, 1H), 4.31 (dd, 1H, J = 2.0, 5.6 Hz), 5.32-5.34 (m, 2H), 5 .66 (d, 1H, J = 8.6 Hz), 5.92 (d, 1H, J = 5.6 Hz), 6.99 (d, 1H, J = 8.2 Hz), 7.19-7.24 (m, 1H), 7.28-7.33 (m, 2H), 7.44-7.47 (m, 2H), 7.52-7.56 (m, 1H), 8.82-8.86 (m, 2H), 9.1 (s, 1H), 10.69 (d, 1H, J = 8.6 Hz); MS (ES+) m/z (relative intensity) 433 (M+H) (100), 455 (M+H+Na) (60); HPLC purity = 99.9 %.

5.3.1.6 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-cyclopropylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 78):

Compound **78** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-cyclopropyl-3-amino-2-hydroxy-3- phenylpropane-1-carboxamide (**38**), similar to procedure of compound **77**.

R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$ + DMSO-d_6) 0.33-0.34 (m, 1H), 0.41-0.43 (m, 1H), 0.59-0.63 (m, 2H), 2.61-2.66 (m, 1H, partially merged with DMSO peak), 2.72-2.73 (m, 1H), 4.32 (d, 1H, J = 5.5 Hz), 5.33-5.39 (m, 3H), 5.65 (d, 1H, J = 8.5 Hz), 5.86-5.88 (m, 1H), 7.19-7.33 (m, 2H), 7.44-7.62 (m, 4H), 8.81-8.86 (m, 2H), 9.14 (s, 1H), 10.62 (d, 1H, J = 7.6 Hz); MS (ES+) m/z (relative intensity) 431 (M+H) (20), 453 (M+H+Na) (100); HPLC purity = 99.8 %.

5.3.1.7 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 79):

Compound **79** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-cyclopentyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**39**), similar to procedure of compound **77**.

R_f 0.5 (10 % MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.37-1.59 (m, 4H), 1.76-1.91 (m, 4H), 2.52 (t, 1H, J = 2.2 Hz), 4.12-4.21 (m, 1H), 4.30-4.31 (m, 1H), 4.49-4.52 (m, 1H), 5.27 (s, 2H), 5.66 (dd, 1H, J = 2.8, 8.2 Hz), 6.69 (d, 1H, J = 7.6 Hz), 7.22-7.34 (m, 3H), 7.45-7.52 (m, 3H), 8.81-8.84 (m, 2H), 9.14 (s, 1H), 10.83 (d, 1H, J = 8.2 Hz) ; MS (ES+) m/z (relative intensity) 459 (M+H) (10), 481 (M+H+Na) (100); HPLC purity = 98.1 %.

5.3.1.8 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-cyclohexylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 80):

Compound **80** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-cyclohexyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**40**), similar to procedure of compound **77**.

R_f 0.7 (10 % MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.08-1.27 (m, 4H), 1.49-1.83 (m, 5H), 2.09-2.10 (m, 1H), 2.52 (t, 1H, J = 2.4 Hz), 3.73-3.76 (m, 1H), 4.51 (bs, 1H), 4.75 (d, 1H, J = 4.5 Hz), 5.27 (d, 2H, J = 2.4 Hz), 5.69 (dd, 1H, J = 2.8, 8.4 Hz), 6.72 (d, 1H, J = 8.5 Hz), 7.18-7.30 (m, 3H), 7.44-7.50 (m, 3H), 8.82 (d, 2H, J = 6.5 Hz), 9.11 (s, 1H), 10.88 (d, 1H, J = 8.4 Hz); MS (ES+) m/z (relative intensity) 473 (M+H) (100), 495 (M+H+Na) (80); HPLC purity = ~ 100 %.

5.3.1.9 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide (Compound 81):

Compound **81** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-phenyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**41**), similar to procedure of compound **77**.

R_f 0.6 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.55 (1H, merged with water peak), 4.46 (d, 1H, J = 5.7 Hz), 5.43'(s, 2H), 5.72 (d, 1H, J = 8.8 Hz), 6.46 (d, 1H, J = 5.7 Hz), 7.06-7.11 (m, 1H), 7.27-7.34 (m, 3H), 7.36-7.51 (m, 4H), 7.66-7.77 (m, 3H), 8.81 (d, 1H, J = 6.5 Hz), 9.0-9.02 (m, 1H), 9.13 (s, 1H), 9.73 (s, 1H), 10.63 (d, 1H, J = 8.8 Hz); MS (ES+) m/z (relative intensity) 466 (M+H) (20), 489 (M+H+Na) (100); HPLC purity = ~ 100 %.

5.3.1.10 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-(4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide (Compound 82):

Compound **82** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-(4'-fluoro)phenyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**42**), similar to procedure of compound **77**.

R_f 0.6 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.48 (t, 1H, J = 2.3 Hz), 4.39 (dd, 1H, J = 2.1, 5.7 Hz), 5.36 (s, 2H), 5.63 (d, 1H, J = 8.9 Hz), 6.38 (d, 1H, J = 5.7 Hz), 7.03-7.09 (m, 2H), 7.24-7.42 (m, 5H), 6.61-7.68 (m, 3H), 8.72 (dd, 1H, J = 1.7, 7.9

Hz), 8.92-8.94 (m, 1H), 9.05 (s, 1H), 9.78 (s, 1H), 10.54 (d, 1H, J = 8.9 Hz); MS (ES+) m/z (relative intensity) 485 (M+H) (30), 507 (M+H+Na) (100); HPLC purity = 98.0 %.

5.3.1.11 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-2-(4-methoxy-phenylcarbamoyl)-1-phenyl-ethyl]-amide (Compound 83):

Compound **83** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-(4'-methoxy) phenyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**44**), similar to procedure of compound **77**.

R_f 0.4 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.50 (t, 1H, J = 2.3 Hz), 3.71 (s, 3H), 4.66-4.69 (m, 1H), 5.13 (d, 1H, J = 5.2 Hz), 5.22 (s, 2H), 5.81 (dd, 1H, J = 2.3, 8.3 Hz), 6.73 (d, 2H, J = 8.8 Hz), 7.16-7.30 (m, 3H), 7.39-7.49 (m, 5H), 8.67 (s, 1H), 8.76-8.80 (m, 2H), 9.09 (s, 1H), 10.90 (d, 1H, J = 8.3 Hz); MS (ES+) m/z (relative intensity) 497 (M+H) (100), 519 (M+H+Na) (60); HPLC purity = 99.1 %.

5.3.1.12 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-benzylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 84):

Compound **84** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-benzyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**46**), similar to procedure of compound **77**.

R_f 0.4 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.54 (s, 1H), 4.04-4.10 (m, 1H), 4.28 (d, 1H, J = 5.5 Hz), 4.42-4.50 (m, 1H), 5.37-5.52 (m, 2H), 5.62 (d, 1H, J = 8.7 Hz), 6.24 (d, 1H, J = 5.7 Hz), 6.87-6.88 (m, 3H), 7.05-7.06 (m, 2H), 7.25-7.41 (m, 5H), 7.65-7.69 (m, 1H), 8.31-8.33 (m, 1H), 8.70 (d, 1H, J = 6.9 Hz), 8.97 (d, 1H, J = 3.2 Hz), 9.1 (s, 1H), 10.56 (d, 1H, J = 8.7 Hz); MS (ES+) m/z (relative intensity) 481 (M+H) (60), 503 (M+H+Na) (100); HPLC purity = 98.2 %.

5.3.1.13 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide (Compound 85):

Compound **85** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-2'-pyridine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**47**), similar to procedure of compound **77**.

R_f 0.5 (EtOAc); ^{1}HNMR (DMSO-d_6) δ 3.48 (s, 1H), 4.51 (d, 1H, J = 5.4 Hz), 5.36 (s, 2H), 5.7 (d, 1H, J = 8.8 Hz), 6.6 (d, 1H, J = 5.4 Hz), 7.09-7.46 (m, 6H), 7.64-7.79 (m, 2H), 8.07 (d, 1H, J = 8.4 Hz), 8.25 (d, 1H, J = 3.6 Hz), 8.74 (d, 1H, J = 7.4 Hz), 8.93 (d, 1H, J = 4.1 Hz), 9.0 (s, 1H), 9.7 (bs, 1H), 10.61 (d, 1H, J = 9.0 Hz); MS (ES+) m/z (relative intensity) 468 (M+H) (15), 490 (M+H+Na) (100); HPLC purity = 99.6 %.

5.3.1.14 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-3-ylcarbamoyl)-ethyl]-amide (Compound 86):

Compound **86** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-3'-pyridine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**48**), similar to procedure of compound **77**.

R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.53 (t, 1H, J = 2.4 Hz), 4.70 (d, 1H, J = 2.3 Hz), 5.24-5.30 (m, 2H), 5.73 (bs, 1H), 5.80 (dd, 1H, J = 2.3, 8.3 Hz), 7.23-7.34 (m, 4H), 7.46-7.51 (m, 3H), 8.17-8.21 (m, 2H), 8.40 (s, 1H), 8.78-8.83 (m, 2H), 8.92 (s, 1H), 9.12 (s, 1H), 10.94 (d, 1H, J = 8.3 Hz); MS (ES+) m/z (relative intensity) 468 (M+H) (50), 490 (M+H+Na) (100); HPLC purity = 99.3 %.

5.3.1.15 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-4-ylcarbamoyl)-ethyl]-amide (Compound 87):

Compound **87** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-4'-pyridine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**49**), similar to procedure of compound **77**.

R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.52 (t, 1H, J = 2.4 Hz), 4.71 (d, 1H, J = 2.7 Hz), 5.26 (d, 2H, J = 2.1 Hz), 5.77 (d, 1H, J = 5.7 Hz), 7.29-7.37 (m, 3H), 7.47-7.52 (m, 5H), 8.34-8.36 (m, 2H), 8.79-8.83 (m, 2H), 8.96 (bs, 1H), 9.13 (s, 1H), 10.92 (d, 1H, J = 8.2 Hz); MS (ES+) m/z (relative intensity) 468 (M+H) (95), 490 (M+H+Na) (100); HPLC purity = 99.9 %.

5.3.1.16 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(thiazol-2-ylcarbamoyl)-ethyl]-amide (Compound 88):

Compound **88** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-2'-thiazole- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**50**), similar to procedure of compound **77**.

R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.51 (t, 1H, *J* = 2.4 Hz), 4.80 (s, 1H), 5.21-5.24 (m, 2H), 5.86 (d, 1H, *J* = 6.5 Hz), 6.33 (bs, 1H), 6.88 (d, 1H, *J* = 3.5 Hz), 7.20-7.35 (m, 4H), 7.44-7.52 (m, 3H), 8.71-8.73 (m, 1H), 8.79-8.81 (m, 1H), 9.11 (s, 1H), 10.69 (bs, 1H), 10.84 (d, 1H, *J* = 8.3 Hz); MS (ES+) m/z (relative intensity) 474 (M+H) (10), 496 (M+H+Na) (100); HPLC purity = 98.9 %.

5.3.1.17 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-piperidin-1-yl-propyl)-amide (Compound 89):

Compound **89** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-piperidine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**51**), similar to procedure of compound **77**.

R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.35-1.65 (m, 6H), 2.49 (t, 1H, *J* = 2.4 Hz), 3.5-3.6 (m, 4H), 4.49 (d, 1H, *J* = 6.4 Hz), 4.73 (d, 1H, *J* = 4.8 Hz), 5.22-5.35 (m, 2H), 5.57 (d, 1H, *J* = 8.2 Hz), 7.26-7.39 (m, 3H), 7.46-7.54 (m, 3H), 8.8-8.9 (m, 2H), 9.10 (s, 1H), 10.66 (d, 1H, *J* = 8.8 Hz); MS (ES+) m/z (relative intensity) 459 (M+H) (20), 481 (M+H+Na) (100); HPLC purity = 92.2 %.

5.3.1.18 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-pyrrolidin-1-yl-propyl)-amide (Compound 90):

Compound **90** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 1-*N*-pyrrolodine- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**53**), similar to procedure of compound **77**.

R_f 0.4 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.85-1.97 (m, 4H), 2.5 (s, 1H), 3.42-3.57 (m, 4H), 4.28 (d, 1H, *J* =- 6.7 Hz), 4.54 (dd, 1H, *J* = 2.5, 6.7 Hz), 5.26 (dd, 2H, *J* = 2.3, 14.8 Hz), 5.61 (dd, 1H, *J* = 2.5, 8.6 Hz), 7.26-7.38 (m, 3H), 7.46-7.52 (m, 3H), 8.81-8.85 (m, 2H), 9.11 (s, 1H), 10.72 (d, 1H, *J* = 8.6 Hz); MS (ES+) m/z (relative intensity) 445 (M+H) (15), 467 (M+H+Na) (100); HPLC purity = 98.6 %.

5.3.1.19 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 91):

Compound **91** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-cyclopentyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**39**), similar to procedure of compound **77**.

R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.20-1.54 (m, 6H), 1.74-1.91 (m, 2H), 2.55 (s, 1H), 4.07-4.19 (m, 2H), 4.49 (s, 1H), 5.21 (s, 2H), 5.65 (d, 1H, J = 7.0 Hz), 6.62 (d, 1H, J = 7.3 Hz), 7.21-7.47 (m, 6H), 8.75 (d, 1H, J = 8.2 Hz), 9.14 (s, 1H), 10.76 (d, 1H, J = 7.8 Hz); MS (ES+) m/z (relative intensity) 493 (M+H) (100), 515 (M+H+Na) (70); HPLC purity = 99.0 %.

5.3.1.20 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-cyclohexylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 92):

Compound **92** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-cyclohexyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**40**), similar to procedure of compound **77**.

R_f 0.4 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.88-1.37 (m, 7H), 1.51-1.55 (m, 2H), 1.81-1.84 (m, 1H), 2.55 (t, 1H, J = 2.2 Hz), 3.70-3.77 (m, 1H), 4.16 (d, 1H, J = 4.9 Hz), 4.48-4.50 (m, 1H), 5.20 (s, 2H), 5.65 (dd, 1H, J = 2.6, 8.1 Hz), 6.57 (d, 1H, J = 8.4 Hz), 7.23-7.46 (m, 6H), 8.74 (d, 1H, J = 8.3 Hz), 9.11 (s, 1H), 10.76 (d, 1H, J = 8.1 Hz); MS (ES+) m/z (relative intensity) 507 (M+H) (40), 529 (M+H+Na) (100); HPLC purity = 98.6 %.

5.3.1.21 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-cyclohexylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 93):

Compound **93** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-phenyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**41**), similar to procedure of compound **77**.

R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.52 (s, 1H), 4.38 (d, 1H, J = 3.8 Hz), 5.26 (s, 2H), 5.63 (d, 1H, J = 8.1 Hz), 6.38 (d, 1H, J = 5.5 Hz), 6.97-7.02 (m,

1H), 7.20-7.42 (m, 7H), 7.60 (d, 2H, J = 7.8 Hz), 7.70 (d, 1H, J = 8.3 Hz), 8.69 (d, 1H, J = 8.3 Hz), 9.02 (s, 1H), 9.65 (s, 1H), 10.45 (d, 1H, J = 8.8 Hz); MS (ES+) m/z (relative intensity) 501 (M+H) (40), 523 (M+H+Na) (100); HPLC purity = 98.8 %.

5.3.1.22 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-(4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide (Compound 94):

Compound **94** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-(4'-fluoro)phenyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**42**), similar to procedure of compound **77**. R_f 0.7 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.54 (s, 1H), 4.39 (bs, 1H), 5.28 (s, 2H), 5.63-5.65 (m, 1H), 6.41 (bs, 1H), 7.07-7.09 (m, 2H), 7.25-7.40 (m, 5H), 7.64-7.73 (m, 3H), 8.71 (d, 1H, J = 8.1 Hz), 9.04 (s, 1H), 9.80 (bs, 1H), 10.46 (d, 1H, J = 9.0 Hz); MS (ES+) m/z (relative intensity) 519 (M+H) (10), 541 (M+H+Na) (100); HPLC purity = 99.6 %.

5.3.1.23 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-2-(4-methoxy-phenylcarbamoyl)-1-phenyl-ethyl]-amide (Compound 95):

Compound **95** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-(4'-methoxy)phenyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**44**), similar to procedure of compound **77**. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.54 (s, 1H), 2.54 (s, 1H), 3.73 (s, 3H), 4.67 (s, 1H), 4.77 (d, 1H, J = 5.2 Hz), 5.17 (s, 2H), 5.77 (d, 1H, J = 7.9 Hz), 6.75 (d, 2H, J = 8.7 Hz), 7.20-7.49 (m, 8H), 8.57 (s, 1H), 8.70 (d, 1H, J = 8.3 Hz), 9.08 (s, 1H), 10.81 (d, 1H, J = 7.9 Hz); MS (ES+) m/z (relative intensity) 531 (M+H) (100), 553 (M+H+Na) (50); HPLC purity = 98.9 %.

5.3.1.24 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-benzylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 96):

Compound **96** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-benzyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**46**), similar to procedure of compound **77**.

R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.57 (t, 1H, *J* = 2.4 Hz), 4.21-4.28 (m, 1H), 4.40 (d, 1H, *J* = 5.3 Hz), 4.52-4.61 (m, 2H), 5.20 (s, 2H), 5.73 (dd, 1H, *J* = 2.4, 8.4 Hz), 6.98-7.46 (m, 12H), 8.68 (d, 1H, *J* = 8.3 Hz), 9.03 (s, 1H), 10.77 (d, 1H, *J* = 8.4 Hz); MS (ES+) m/z (relative intensity) 515 (M+H) (40), 537 (M+H+Na) (100); HPLC purity = 98.4 %.

5.3.1.25 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide (Compound 97):

Compound **97** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-2'-pyridine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**47**), similar to procedure of compound **77**.

R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.52 (s, 1H), 4.48 (d, 1H, *J* = 5.5 Hz), 5.26 (s, 2H), 5.67 (d, 1H, *J* = 9.0 Hz), 6.57 (d, 1H, *J* = 5.5 Hz), 7.06-7.10 (m, 1H), 7.21-7.43 (m, 5H), 7.69-7.78 (m, 2H), 8.05 (d, 1H, *J* = 8.3 Hz), 8.24 (d, 1H, *J* = 4.3 Hz), 8.71 (d, 1H, *J* = 8.2 Hz), 9.02 (s, 1H), 9.68 (s, 1H), 10.50 (d, 1H, *J* = 9.0 Hz); MS (ES+) m/z (relative intensity) 502 (M+H) (50), 524 (M+H+Na) (100); HPLC purity = 96.1 %.

5.3.1.26 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-3-ylcarbamoyl)-ethyl]-amide (Compound 98):

Compound **98** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-3'-pyridine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**48**), similar to procedure of compound **77**.

R_f 0.4 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.53 (t, 1H, *J* = 2.3 Hz), 4.42-4.45 (m, 1H), 5.27 (s, 2H), 5.65 (dd, 1H, *J* = 1.5, 8.9 Hz), 6.48 (d, 1H, *J* = 5.7 Hz), 7.25-

7.43 (m, 6H), 7.72 (d, 1H, *J* = 8.3Hz), 8.03-8.06 (m, 1H), 8.21 (d, 1H, *J* = 4.0 Hz), 8.70 (d, 1H, *J* = 8.3 Hz), 8.78 (bs 1H), 9.04 (s, 1H), 10.0 (s, 1H), 10.49 (d, 1H, *J* = 8.9 Hz); MS (ES+) m/z (relative intensity) 502 (M+H) (50), 524 (M+H+Na) (100); HPLC purity = 99.4 %.

5.3.1.27 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(thiazol-2-ylcarbamoyl)-ethyl]-amide (Compound 99):

Compound **99** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-2'-thiazole- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**50**), similar to procedure of compound **77**.

R_f 0.4 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.55 (t, 1H, *J* = 2.3 Hz), 4.78 (s, 1H), 5.11-5.24 (m, 2H), 5.80-5.83 (m, 1H), 5.99 (bs, 1H), 6.89 (d, 1H, *J* = 3.5 Hz), 7.22-7.50 (m, 7H), 8.67 (d, 1H, *J* = 8.3 Hz), 9.1 (s, 1H), 10.48 (bs, 1H), 10.76 (d, 1H, *J* = 8.2 Hz); MS (ES+) m/z (relative intensity) 508 (M+H) (20), 530 (M+H+Na) (50); HPLC purity = 97.7 %.

5.3.1.28 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-piperidin-1-yl-propyl)-amide (Compound 100):

Compound **100** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-piperidine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**51**), similar to procedure of compound **77**.

R_f 0.7 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.57-1.71 (m, 6H), 2.53 (t, 1H, *J* = 2.5 Hz), 3.49-3.60 (m, 4H), 4.47 (d, 1H, *J* = 6.4 Hz), 4.72-4.74 (m, 1H), 5.10-5.29 (m, 2H), 5.54 (d, 1H, *J* = 9.0 Hz), 7.27-7.53 (m, 6H), 8.74 (d, 1H, *J* = 8.3 Hz), 9.07 (s, 1H), 10.56 (d, 1H, *J* = 9.0 Hz); MS (ES+) m/z (relative intensity) 493 (M+H) (30), 515 (M+H+Na) (100); HPLC purity = 98.8 %.

5.3.1.29 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-3-oxo-1-phenyl-propyl)-amide (Compound 101):

Compound **101** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 1-*N*-morpholine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**52**), similar to procedure of compound **77**.

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.40-3.50 (m, 9H), 4.76 (s, 1H), 5.20 (d, 1H, J = 6.2 Hz), 5.30 (s, 2H), 5.42 (d, 1H, J = 8.4 Hz), 7.24-7.41 (m, 5H), 7.69 (d, 1H, J = 8.3 Hz), 8.71 (d, 1H, J = 8.3 Hz), 9.06 (s, 1H), 10.37 (d, 1H, J = 8.4 Hz); MS (ES+) m/z (relative intensity) 495 (M+H) (20), 517 (M+H+Na) (100); HPLC purity = 98.4 %.

5.3.1.30 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-cyclopropylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 102):

Compound **102** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-cyclopropyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide (**38**), similar to procedure of compound **77**.

R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.33-0.48 (m, 2H), 0.65-0.74 (m, 2H), 2.57-2.69 (m, 2H), 3.85 (bs, 1H), 4.43-4.49 (m, 1H), 5.21 (s, 2H), 5.62-5.65 (m, 1H), 6.79 (bs, 1H), 7.26-7.44 (m, 5H), 8.51 (d, 1H, J = 7.2 Hz), 9.12 (s, 1H), 10.62 (d, 1H, J = 8.0 Hz); MS (ES+) m/z (relative intensity) 483 (M+H) (10), 505 (M+H+Na) (100); HPLC purity = 95.1 %.

5.3.1.31 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 103):

Compound **103** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-cyclopentyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**39**), similar to procedure of compound **77**.

R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.10-1.18 (m, 3H), 1.26-1.55 (m, 4H), 1.60-1.65 (m, 1H), 2.49 (s, 1H), 4.02 (d, 1H, J = 4.8 Hz), 4.07-4.14 (m, 1H), 4.41 (s, 1H), 5.13 (s, 2H), 5.58 (d, 1H, J = 6.0 Hz), 6.57 (d, 1H, J = 7.6 Hz), 7.15-7.27

(m, 3H), 7.35-7.38 (m, 2H), 8.43 (d, 1H, J = 7.2 Hz), 9.04 (s, 1H), 10.62 (d, 1H, J = 8.1 Hz); MS (ES+) m/z (relative intensity) 511 (M+H) (10), 533 (M+H+Na) (100); HPLC purity = 93.1 %.

5.3.1.32 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-cyclohexylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 104):

Compound **104** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-cyclohexyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**40**), similar to procedure of compound **77**.

R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 0.99-1.22 (m, 5H), 1.45-1.60 (m, 5H), 3.40-3.56 (m, 2H), 4.16 (dd, 1H, J = 2.0, 5.4 Hz), 5.31 (d, 2H, J = 2.0 Hz), 5.48 (d, 1 H, J = 8.8 Hz), 6.06 (d, 1H, J = 5.4 Hz), 7.20-7.37 (m, 6H), 8.65 (d, 1H, J = 7.7 Hz), 9.07 (s, 1H), 10.34 (d, 1H, J = 8.8 Hz); MS (ES+) m/z (relative intensity) 525 (M+H) (100), 547 (M+H+Na) (100); HPLC purity = 99.0 %.

5.3.1.33 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide (Compound 105):

Compound **105** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-phenyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide (**41**), similar to procedure of compound **77**.

R_f 0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.54 (s, 1H), 4.40 (s, 1H), 5.29 (s, 2H), 5.65 (d, 1H, J = 8.8 Hz), 6.39 (d, 1H, J = 5.2 Hz), 7.02-7.04 (m, 1H), 7.23-7.44 (m, 7H), 7.61 (d, 2H, J = 7.8 Hz), 8.64 (d, 1H, J = 7.8 Hz), 9.06 (s, 1H), 9.63 (s, 1H), 10.42 (d, 1H, J = 8.8 Hz); MS (ES+) m/z (relative intensity) 519 (M+H) (40), 541 (M+H+Na) (100); HPLC purity = 99.8 %.

5.3.1.34 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-(4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide (Compound 106):

Compound **106** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-(4'-fluoro)phenyl- 3- amino-2-

hydroxy-3- phenylpropane-1-carboxamide (**42**), similar to procedure of compound **77**.

R_f 0.5 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.55 (s, 1H), 4.40 (s, 1H), 5.29 (s, 2H), 5.63-5.65 (m, 1H), 6.41 (d, 1H, J = 5.4 Hz), 7.05-7.11 (m, 2H), 7.24-7.42 (m, 5H), 7.63-7.66 (m, 2H), 8.63 (m, 1H), 9.06 (s, 1H), 9.78 (s, 1H), 10.42 (d, 1H, J = 8.5 Hz); MS (ES+) m/z (relative intensity) 537 (M+H) (30), 559 (M+H+Na) (100); HPLC purity = 98.6 %.

5.3.1.35 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-(3-chloro-4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide (Compound 107):

Compound **107** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-(3'-chloro-4'-fluoro)phenyl - 3- amino-2-hydroxy -3- phenylpropane-1-carboxamide (**45**), similar to procedure of compound **77**.

R_f 0.7 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.54 (t, 1H, J = 2.3 Hz), 4.40 (dd, 1H, J = 2.2, 5.7 Hz), 5.28-5.29 (m, 2H), 5.62-5.65 (m, 1H), 6.49 (d, 1H, J = 5.7 Hz), 7.22-7.42 (m, 6H), 7.58-7.63 (m, 1H), 7.92-7.96 (m, 1H), 8.62 (d, 1H, J = 7.7 Hz), 9.05 (s, 1H), 9.97 (s, 1H), 10.42 (d, 1H, J = 9.0 Hz); MS (ES+) m/z (relative intensity) 571 (M+H) (100), 593 (M+H+Na) (90); HPLC purity = 99.5 %.

5.3.1.36 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-benzylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 108):

Compound **108** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-benzyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**46**), similar to procedure of compound **77**.

R_f 0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.54 (s, 1H), 4.05-4.08 (m, 1H), 4.26 (s, 1H), 4.39-4.44 (m, 1H), 5.30 (s, 2H), 5.58-5.60 (m, 1H), 6.24 (s, 1H), 6.93-7.05 (m, 5H), 7.24-7.50 (m, 5H), 8.32 (s, 1H), 8.57 (d, 1H, J = 7.8 Hz), 9.09 (s, 1H), 10.41 (d, 1H, J = 8.6 Hz); MS (ES+) m/z (relative intensity) 533 (M+H) (90), 555 (M+H+Na) (100); HPLC purity = 99.0 %.

5.3.1.37 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide (Compound 109):

Compound **109** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-2'-pyridine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**47**), similar to procedure of compound **77**.

R_f 0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.54 (t, 1H, J = 2.3 Hz), 4.48 (dd, 1H, J = 1.9, 5.6 Hz), 5.27-5.28 (m, 2H), 5.65-5.68 (m, 1H), 6.60 (d, 1H, J = 5.6 Hz), 7.07-7.11 (m, 1H), 7.22-7.43 (m, 5H), 7.38-7.79 (m, 1H), 8.04 (d, 1H, J = 8.3 Hz), 8.25 (d, 1H, J = 3.7 Hz), 8.67 (d, 1H, J = 7.8 Hz), 9.07 (s, 1H), 9.67 (s, 1H), 10.46 (d, 1H, J = 9.0 Hz); MS (ES+) m/z (relative intensity) 542 (M+H+Na) (100); HPLC purity = 97.6 %.

5.3.1.38 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-3-ylcarbamoyl)-ethyl]-amide (Compound 110):

Compound **110** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-3'-pyridine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**48**), similar to procedure of compound **77**.

R_f 0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.54 (s, 1H), 4.42 (d, 1H, J = 5.3 Hz), 5.27 (s, 2H), 5.64 (d, 1H, J = 8.6 Hz), 6.48 (d, 1H, J = 5.3 Hz), 7.24-7.42 (m, 6H), 8.02-8.05 (m, 1H), 8.21-8.22 (m, 1H), 8.62 (d, 1H, J = 7.7 Hz), 8.78 (bs, 1H), 9.05 (s, 1H), 9.98 (s, 1H), 10.42 (d, 1H, J = 8.6 Hz); MS (ES+) m/z (relative intensity) 520 (M+H) (40), 542 (M+H+Na) (100).

5.3.1.39 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-4-ylcarbamoyl)-ethyl]-amide (Compound 111):

Compound **111** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-4'-pyridine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**49**), similar to procedure of compound **77**.

R_f 0.6 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.54 (s, 1H), 4.43 (dd, 1H, J = 2.1, 5.7 Hz), 5.27 (d, 2H, J = 1.8 Hz), 5.63-5.66 (m, 1H), 6.48 (d, 1H, J = 5.7 Hz), 7.21-

7.42 (m, 5H), 7.67 (d, 2H, J = 6.2 Hz), 8.36 (d, 2H, J = 5.7 Hz), 8.62 (d, 1H, J = 7.7 Hz), 9.04 (s, 1H), 10.10 (s, 1H), 10.41 (d, 1H, J = 9.0 Hz); MS (ES+) m/z (relative intensity) 520 (M+H) (100), 542 (M+H+Na) (70); HPLC purity = 97.5 %.

5.3.1.40 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(thiazol-2-ylcarbamoyl)-ethyl]-amide (Compound 112):

Compound **112** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-2'-thiazole- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**50**), similar to procedure of compound **77**.

R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.48-2.49 (m, 1H), 4.67 (s, 1H), 5.19-5.22 (m, 2H), 5.79-5.82 (m, 1H), 6.27 (bs, 1H), 6.80 (d, 1H, J = 3.5 Hz), 7.01 (d, 1H, J = 3.5 Hz), 7.15-7.41 (m, 5H), 8.38 (d, 1H, J = 7.2 Hz), 9.03 (s, 1H), 10.61 (d, 1H, J = 8.4 Hz); MS (ES+) m/z (relative intensity) 526 (M+H) (25), 548 (M+H+Na) (100); HPLC purity = 97.2 %.

5.3.1.41 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-piperidin-1-yl-propyl)-amide (Compound 113):

Compound **113** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 1-*N*-piperidine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**51**), similar to procedure of compound **77**.

R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.44-1.78 (m, 5H), 1.98-1.99 (m, 1H), 2.55 (s, 1H), 3.49-3.61 (m, 4H), 4.48 (d, 1H, J = 6.0 Hz), 4.72-4.73 (m, 1H), 5.08-5.24 (m, 2H), 5.51-5.54 (m, 1H), 7.24-7.51 (m, 5H), 8.50 (d, 1H, J = 7.2 Hz), 9.06 (s, 1H), 10.47 (d, 1H, J = 9.0 Hz); MS (ES+) m/z (relative intensity) 511 (M+H) (40), 533 (M+H+Na) (100); HPLC purity = 96 %.

5.3.1.42 7-Methyl-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide (Compound 114):

Compound **114** was prepared from amidation of 7-methyl-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76d** with 1-*N*-2'-pyridine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**47**), similar to procedure of compound **77**.

R_f 0.3 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 2.65 (s, 3H), 3.47-3.48 (m, 1H), 4.47-4.48 (m, 1H), 5.33 (s, 2H), 5.64-5.67 (m, 1H), 6.56 (d, 1H, J = 5.6 Hz), 7.05-7.09 (m, 1H), 7.21-7.43 (m, 5H), 7.51 (d, 1H, J = 8.1 Hz), 7.73-7.78 (m, 1H), 8.04 (d, 1H, J = 8.2 Hz), 8.24 (d, 1H, J = 4.3 Hz), 8.59 (d, 1H, J = 8.1 Hz), 8.97 (s, 1H), 9.67 (s, 1H), 10.62 (d, 1H, J = 9.0 Hz); MS (ES+) m/z (relative intensity) 504 (M+H) (100); HPLC purity = 99.6 %.

5.3.1.43 2-Hydroxy-3-[(4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthydrine-3-carbonyl)-amino]-3-phenyl-propionic acid ethyl ester (Compound 115):

Compound **115** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with phenylisoserine ethyl ester, similar to procedure of compound **77**.

R_f 0.6 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.23 (t, 3H, J = 7.1 Hz), 2.44 (s, 1H), 3.44 (d, 1H, J = 5.2 Hz), 4.16-4.23 (m, 2H), 4.47 (d, 1H, J = 2.1 Hz), 5.19 (dd, 2H, J = 2.1, 7.4 Hz), 5.63-5.66 (m, 1H), 7.19-7.43 (m, 6H), 8.74-8.77 (m, 2H), 9.08 (s, 1H), 10.65 (d, 1H, J = 8.6 Hz); MS (ES+) m/z (relative intensity) 420 (M+H) (20), 442 (M+H+Na) (100); HPLC purity = 98.6 %.

5.3.1.44 6-Fluoro-4-oxo-1-prop-2-ynyl-7-pyrrolidin-1-yl-1,4-dihydro-[1,8]naphthydrine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 116):

To a solution of 0.50 g (0.97 mmol) of **103** and 0.29 g (2.93 mmol) of triethylamine in 20 ml of acetonitrile was added 0.10 g (1.46 mmol) of pyrrolidine. The resulting mixture was refluxed for 3h. The resulting mixture was concentrated, diluted with water and extracted in dichloromethane (50 ml). The organic layer was dried over sodium sulphate and concentrated to dryness to afford a crude product, which was chromatographed on silica gel with 2%MeOH/DCM to afford compound **116**.

R_f 0.4 (8% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.24-2.03 (m, 12H), 2.45 (t, 1H, J = 2.4 Hz), 3.80-3.81 (m, 4H), 4.16-4.19 (m, 1H), 4.49-4.50 (m, 1H), 5.04-5.05 (m, 2H), 5.61 (dd, 1H, J = 2.6, 8.1 Hz), 6.71 (d, 1H, J = 7.8 Hz), 7.22-7.47 (m, 5H), 8.03 (d, 1H, J = 12.8 Hz), 8.81 (s, 1H), 11.09 (d, 1H, J = 8.1 Hz); MS (ES+) m/z (relative intensity) 546 (M+H) (70), 568 (M+H+Na) (100); HPLC purity = 98.5 %.

5.3.1.45 6-Fluoro-7-(3-methyl-piperidin-1-yl)-4-oxo-1-prop-2-ynyl-1,4-dihydro-1,8] naphthydrine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 117):

Compound **117** was prepared from alkylation of **103** with 3-methyl piperidine, similar to procedure of compound **116**.

R_f 0.5 (8% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.97 (d, 3H, J = 6.5 Hz), 1.23-1.91 (m, 13H), 2.45-2.46 (m, 1H), 2.74-2.82 (m, 1H), 3.06-3.14 (m, 1H), 4.14-4.21 (m, 1H), 4.36-4.49 (m, 4H), 5.02 (s, 2H), 5.62 (dd, 1H, J = 2.0, 7.8 Hz), 6.70 (d, 1H, J = 7.8 Hz), 7.22-7.47 (m, 5H), 8.06 (d, 1H, J = 13.7 Hz), 8.82 (s, 1H), 11.03 (d, 1H, J = 8.1 Hz); MS (ES+) m/z (relative intensity) 574 (M+H) (100), 596 (M+H+Na) (70); HPLC purity = 99.6 %.

5.3.1.46 6-Fluoro-7-(3-methyl-piperidin-1-yl)-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthydrine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide (Compound 118):

Compound **118** was prepared from alkylation of **105** with 3-methyl piperidine, similar to procedure of compound **116**.

R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.96 (d, 3H, J = 6.2 Hz), 1.24-1.26 (m, 2H), 1.70-1.92 (m, 3H), 2.44 (s, 1H), 2.74-2.82 (m, 1H), 3.05-3.14 (m, 1H), 4.35-4.45 (m, 2H), 4.69 (s, 1H), 4.99 (s 2H), 5.24 (s, 1H), 5.72 (d, 1H, J = 3.9 Hz), 7.04-7.34 (m, 6H), 7.49-7.55 (m, 4H), 8.05 (d, 1H, J = 13.7 Hz), 8.73-8.80 (m, 2H), 11.15 (d, 1H, J = 7.5 Hz); MS (ES+) m/z (relative intensity) 582 (M+H) (80), 604 (M+H+Na) (100); HPLC purity = 99.1 %.

5.3.1.47 4-Oxo-1-thiazol-2-yl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide (Compound 119):

Compound **119** was prepared from amidation of 1-thiazolyl-1,8-naphthyridine-3-carboxylic acid **76e** with 1-*N*-2'-pyridine-3-amino-2-hydroxy-3- phenylpropane-1-carboxamide (**47**), similar to procedure of compound **77**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 4.51 (d, 1H, J = 5.4 Hz), 5.70 (d, 1H, J = 8.8 Hz), 6.65 (d, 1H, J = 5.5 Hz), 7.06-7.44 (m, 6H), 7.74-7.80 (m, 4H), 8.05 (d, 1H, J = 8.2 Hz), 8.25 (d, 1H, J = 4.8 Hz), 8.82 (d, 1H, J = 1.7 Hz), 9.0 (d, 1H, J = 1.7 Hz), 9.72 (s, 1H), 9.92 (s, 1H), 10.45 (d, 1H, J = 8.9 Hz); MS (ES+) m/z (relative intensity) 513 (M+H) (100); HPLC purity = 98.4 %.

5.3.1.48 7-Chloro-6-fluoro-4-oxo-1-thiazol-2-yl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (Compound 120):

Compound **120** was prepared from amidation of 7-Chloro-6-fluoro-1-thiazolyl-1,8-naphthyridine-3-carboxylic acid **76f** with 1-*N*-cyclopentyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**39**), similar to procedure of compound **77**.

R_f 0.4 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.22-1.75 (m, 8H), 3.94-4.01 (m, 1H), 4.19 (dd, 1H, J = 2.3, 5.4 Hz), 5.49-5.52 (m, 1H), 6.10 (d, 1H, J = 5.4 Hz), 7.22-7.38 (m, 5H), 7.49 (d, 1H, J = 7.8 Hz), 7.82-7.86 (m, 2H), 8.79 (d, 1H, J = 7.8 Hz), 9.88 (s, 1H), 10.22 (d, 1H, J = 8.7 Hz); MS (ES+) m/z (relative intensity) 556 (M+H) (60), 578 (M+H+Na) (100); HPLC purity = 93.2 %.

5.3.1.49 7-Chloro-6-fluoro-4-oxo-1-thiazol-2-yl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-piperidin-1-yl-propyl)-amide (Compound 121):

Compound **121** was prepared from amidation of 7-Chloro-6-fluoro-1-thiazolyl-1,8-naphthyridine-3-carboxylic acid **76f** with 1-*N*-piperidine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**51**), similar to procedure of compound **77**.

R_f 0.7 (10 % MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.25 (s, 1H), 1.61-1.68 (m, 5H), 3.52-3.58 (m, 4H), 4.53 (s, 1H), 4.75 (s, 1H), 5.60 (d, 1H, J = 8.9 Hz), 7.22-7.34 (m, 6H), 7.26-7.54 (m, 6H), 7.72 (s, 1H), 8.58 (d, 1H, J = 7.1 Hz), 10.15 (s, 1H), 10.27 (d, 1H,

J = 8.5 Hz); MS (ES+) m/z (relative intensity) 556 (M+H) (70), 578 (M+H+Na) (100); HPLC purity = 93.4 %.

5.3.1.50 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-1-phenyl-ethyl)-amide (Compound 122):

Compound **122** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with DL-*N*-cyclopentyl 3-amino-3-phenyl propionamide (**60**), similar to procedure of compound **77**.

R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.28-1.23 (m, 2H), 1.49-1.87 (m, 6H), 2.53 (m, 1H), 2.78-2.81 (m, 2H), 4.18 (m, 1H), 5.28 (s, 2H), 5.61 (dd, 1H, J = 6.63 Hz), 5.88 (d, 1H, J = 7.1 Hz), 7.24-7.50 (m, 6H), 8.79-8.82 (2H, m), 9.18 (s, 1H),10.61 (d, 1H, J = 8.1 Hz); MS (ES+) 443 (M+H), m.p. 193-195°C.

5.3.1.51 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclohexylcarbamoyl-1-phenyl-ethyl)-amide (Compound 123):

Compound **123** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with DL-*N*-cyclohexyl 3-amino-3-phenyl propionamide (**61**), similar to procedure of compound **77**.

R_f 0.3 (7% Acetone/$CHCl_3$); ^{1}HNMR ($CDCl_3$) δ 0.87-1.77 (m, 10H), 2.52 (m, 1H), 2.80 (m, 2H), 3.64-3.75 (m, 1H), 5.28 (s, 2H), 5.70 (m, 2H), 7.22-7.50 (m, 6H), 8.81-8.83 (m, 2H), 9.17 (s, 1H), 10.64 (d, 1H, J = 8.1Hz); MS (ES+) 457 (M+H), m.p. 108-210°C.

5.3.1.52 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (1-phenyl-2-phenylcarbamoyl-ethyl)-amide (Compound 124):

Compound **124** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with DL-3-amino-3,*N*-diphenyl-propionamide (**62**), similar to procedure of compound **77**.

R_f 0.4 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.53 (s, 1H), 2.97-3.14 (m, 2H), 5.22-5.35 (m, 2H), 5.72 (q, 1H, J = 7.9, 13.6 Hz), 7.03-7.07 (m, 1H), 7.18-7.60 (m, 10H), 8.24 (s, 1H), 8.78-8.82 (m, 2H), 9.20 (s, 1H), 10.68 (d, 1H, J = 7.65 Hz); MS (ES+) 451 (M+H), m.p. 185-187°C.

5.3.1.53 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide (Compound 125):

Compound **125** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with DL-*N*-2'-pyridine-3-amino-3-phenyl propionamide (**63**), similar to procedure of compound **77**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO) δ 2.97-3.09 (m, 2H), 3.49-3.51 (m, 1H), 5.40 (d, 2H, *J* = 2.37 Hz), 5.60 (q, 1H, *J* = 7.59, 14.8), 7.03-7.05 (m, 1H), 7.21-7.26 (m, 1H), 7.32-7.43 (m, 4H), 7.64-7.74 (m, 2H), 8.02 (d, 1H, *J* = 8.4 Hz), 8.26 (d, 1H, *J* = 3.84 Hz), 8.70 (dd, 1H, *J* = 1.86 Hz), 8.94-8.96 (m, 1H), 9.12 (1H, s), 10.35 (d, 1H, *J* = 8.37),10.56 (s, 1H); MS (ES+) 452 (M+H), m.p. 228-230°C.

5.3.1.54 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-1-phenyl-ethyl)-amide (Compound 126):

Compound **126** was prepared from amidation of 7-Chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with DL-*N*-cyclopentyl 3-amino-3-phenyl propionamide (**60**), similar to procedure of compound **77**.

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.20-1.26 (m, 2H), 1.51-1.59 (m, 4H), 1.79-1.87 (m, 2H), 2.54-2.56 (m, 1H), 2.78 (d, 2H, *J* = 6.61), 4.17 (q, 1H, *J* = 6.7, 13.5), 5.21-5.22 (m, 2H), 5.60 (q, 1H, *J* = 6.7, 14.5 Hz), 5.75 (d, 1H, *J* = 7.37), 7.22-7.46 (m, 5H), 8.73 (d, 1H, *J* = 8.3), 9.15 (s, 1H), 10.57 (d, 1H, *J* = 8.01 Hz); MS (ES+) 477 (M+H), m.p. 208-210°C.

5.3.1.55 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (1-phenyl-2-phenylcarbamoyl-ethyl)-amide (Compound 127):

Compound **127** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with DL-3-amino-3,*N*-diphenyl-propionamide (**62**), similar to procedure of compound **77**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.56 (s, 1H), 3.00-3.07 (m, 2H), 5.21 (s, 2H), 5.71 (d, 1H, *J* = 6.1), 7.02-7.51 (m, 11H), 8.16 (s, 1H), 8.70 (d, 1H, *J* = 8.3), 9.17 (s, 1H), 10.59 (d, 1H, *J* = 8.0 Hz); MS (ES+) 485 (M+H), m.p. 220-222°C.

5.3.1.56 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide (Compound 128):

Compound **128** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with DL-*N*-2'-pyridine-3-amino-3-phenyl propionamide (**63**), similar to procedure of compound **77**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO) δ 2.96-3.12 (m, 2H), 3.57 (t, 1H, J = 2.3 Hz), 5.30-5.31(m, 1H), 5.60 (q, 1H, J = 7.3, 14.7), 7.05(m, 1H), 7.24-7.42 (m, 5H), 7.69-7.74 (m, 2H), 8.01(d, 1H, J = 8.34 Hz), 8.27(d, 1H, J = 3.6), 8.67 (d, 1H, J = 8.31Hz), 9.08 (s, 1H), 9.10 (s, 1H), 10.27 (d, 1H, J = 8.34 Hz), 10.58 (s, 1H); MS (ES+) 486 (M+H), m.p. 208-210°C.

5.3.1.57 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-1-phenyl-ethyl)-amide (Compound 129):

Compound **129** was prepared from amidation of 7-Chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with DL-*N*-cyclopentyl 3-amino-3-phenyl propionamide (**60**), similar to procedure of compound **77**.

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.19-1.87 (m, 8H), 2.56 (d, 1H, J = 2.5 Hz), 2.76-2.78 (m, 2H), 4.16 (q, 1H, J = 6.9, 13.6), 5.20 (s, 2H), 5.58 (q, 1H, J = 6.7 Hz, 14.31Hz), 5.69 (d, 1H, J = 7.26Hz), 7.22-7.42 (m, 5H), 8.50 (d, 1H, J = 7.32 Hz), 9.15 (s, 1H), 10.52 (d, 1H, J = 8.07 Hz); MS (ES+) 495 (M+H), m.p. 208-210°C.

5.3.1.58 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclohexylcarbamoyl-1-phenyl-ethyl)-amide (Compound 130):

Compound **130** was prepared from amidation of 7-Chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with DL-*N*-cyclohexyl 3-amino-3-phenyl propionamide (**61**), similar to procedure of compound **77**.

R_f 0.3 (7% Acetone/$CHCl_3$); ^{1}HNMR ($CDCl_3$) δ 0.94-1.30 (m, 6H), 1.5-1.73 (m, 4H), 2.5 (q, 1H, J = 2.5 Hz), 2.75-2.77 (m, 2H), 3.72-3.75 (m, 1H), 5.20 (d, 2H, J = 2.5 Hz), 5.51-5.60 (m, 2H), 7.22-7.42 (m, 5H), 8.51 (d, 1H, J = 7.32 Hz), 9.14 (s, 1H), 10.57 (d, 1H, J = 8.1 Hz); MS (ES+) 509 (M+H), m.p. 179-181°C.

5.3.1.59 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (1-phenyl-2-phenylcarbamoyl-ethyl)-amide (Compound 131):

Compound **131** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with DL-3-amino-3,*N*-diphenyl-propionamide (**62**), similar to procedure of compound **77**.

R_f 0.6 (7% MeOH/DCM); ^{1}HNMR (DMSO) δ 2.85-3.01 (m, 2H), 3.56 (s, 1H), 5.30 (s, 1H), 5.59 (q, 1H, *J* = 7.1, 14.2 Hz), 6.98 (t, 1H, *J* = 7.2 Hz), 7.11-7.13 (m, 3H), 7.23 – 7.40 (m, 5H), 7.50 (d, 2H, *J* = 7.8 Hz), 8.62 (d, 1H, *J* = 7.8 Hz), 9.1 (1H, s), 9.98 (s, 1H), 10.28 (d, 1H, *J* = 8.2 Hz); MS (ES+) 503 (M+H), m.p. 171-173°C.

5.3.1.60 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide (Compound 132):

Compound **132** was prepared from amidation of 7-chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with DL-*N*-2'-pyridine-3-amino-3-phenyl propionamide (**63**), similar to procedure of compound **77**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO) δ 2.95-3.10 (m, 2H), 3.56 (t, 1H, *J* = 2.14 Hz), 5.30-5.31 (d, 2H, *J* = 2.1 Hz), 5.58 (q, 1H, *J* = 7.4, 14.2 Hz), 7.04 (q, 1H, *J* = 5.2, 6.8 Hz), 7.22 (t, 1H, *J* = 7.14 Hz), 7.30-7.40 (m, 4H), 7.69 (m, 1H), 8.0 (d, 1H, *J* = 8.34 Hz), 8.25 (d, 1H, *J* = 4.14 Hz), 8.60 (d, 1H, *J* = 7.8 Hz), 9.10 (s, 1H), 10.23 (d, 1H, *J* = 8.34 Hz), 10.56 (s, 1H); MS (ES+) 504 (M+H), m.p. 196-198°C.

5.3.1.61 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (cyclopentylcarbamoyl-phenyl-methyl)-amide (Compound 133):

Compound **133** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 2-amino-*N*-cyclopentyl-2-phenyl acetamide (**69**), similar to procedure of compound **77**.

R_f 0.4 (2% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.25-1.60 (m, 6H), 1.92-1.97 (m, 2H), 2.52 (d, 2H, *J* = 2.3 Hz), 4.22 (d, 2H, *J* = 6.9 Hz), 5.27 (q, 2H, *J* = 2.4, 4.8 Hz), 5.61(d, 1H, *J* = 6.9 Hz), 5.83 (d, 1H, *J* = 6.7 Hz), 7.30-7.51 (m, 5H), 8.81 (d, 1H, *J* = 6.6 Hz), 9.16 (s, 1H), 10.77 (d, 1H, *J* = 6.5 Hz); MS (ES+) 429 (M+H), m.p. 215-217°C.

5.3.1.62 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (cyclohexylcarbamoyl-phenyl-methyl)-amide (Compound 134):

Compound **134** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 2-amino-*N*-cyclohexyl-2-phenyl acetamide (**70**), similar to procedure of compound **77**.

R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}HNMR ($CDCl_3$) δ 1.02-1.62 (m, 7H), 1.63-2.02 (m, 3H), 2.51 (d, 1H, J = 2.5 Hz), 2.78-2.80 (m, 1H), 3.75-3.81 (m, 1H), 5.20-5.28 (m, 2H), 5.61 (d, 1H, J = 7.09 Hz), 5.70 (d, 2H, J = 8.3 Hz), 7.26-7.51 (m, 6H), 8.81 (d, 2H, J = 6.7 Hz), 9.16 (s, 1H), 10.66 (d, 1H, J = 6.21 Hz); MS (ES+) 443 (M+H), m.p. 206-208°C.

5.3.1.63 4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (phenyl-phenylcarbamoyl-methyl)-amide (Compound 135):

Compound **135** was prepared from amidation of 1-propargyl-1,8-naphthyridine-3-carboxylic acid **76a** with 2-amino-2,*N*-diphenyl acetamide (**71**), similar to procedure of compound **77**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO) δ 3.51 (s, 1H), 5.41 (bs, 2H), 5.89 (d, 1H, J = 7.4 Hz), 7.04 (s, 1H), 7.29-7.68 (m, 10H), 8.73 (d, 1H, J = 7.35 Hz), 8.96 (s, 1H), 9.15 (s, 1H), 10.51 (s, 1H), 10.73 (d, 1H, J = 5.82 Hz); MS (ES+) 437 (M+H), m.p. 187-189°C.

5.3.1.64 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (cyclopentylcarbamoyl-phenyl-methyl)-amide (Compound 136):

Compound **136** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 2-amino-*N*-cyclopentyl-2-phenyl acetamide (**69**), similar to procedure of compound **77**.

R_f 0.3 (2% MeOH/DCM); ^{1}HNMR (DMSO) δ 1.11-1.82 (m, 8H), 3.52-3.54 (m, 1H), 3.93 (s, 1H), 5.27-5.34 (m, 2H), 5.65 (s, 1H), 7.27-7.40 (m, 5H), 7.70 (d, 1H, J = 9.75 Hz), 8.38 (d, 1H, J = 9.36 Hz), 8.69 (d, 1H, J = 7.02 Hz), 9.09 (s, 1H), 10.52 (s, 1H); MS (ES+) 463 (M+H), m.p. 191-193°C.

5.3.1.65 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (cyclohexylcarbamoyl-phenyl-methyl)-amide (Compound 137):

Compound **137** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 2-amino-*N*-cyclohexyl-2-phenyl acetamide (**70**), similar to procedure of compound **77**.

R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}HNMR (DMSO) δ 0.83-1.26 (m, 5H), 1.49-1.78 (m, 5H), 3.55 (s, 2H), 5.30 (d, 2H, *J* = 1.83 Hz), 5.68 (d, 1H, *J* = 7.8 Hz), 7.16-7.43 (m, 5H), 7.71 (d, 1H, *J* = 8.2 Hz), 8.32 (d, 1H, *J* = 7.74 Hz), 8.69 (d, 1H, *J* = 8.4 Hz), 9.09 (s, 1H), 10.52 (d, 1H, *J* = 7.8 Hz); MS (ES+) 477 (M+H), m.p. > 250°C.

5.3.1.66 7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (phenyl-phenylcarbamoyl-methyl)-amide (Compound 138):

Compound **138** was prepared from amidation of 7-chloro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76b** with 2-amino-2,*N*-diphenyl acetamide (**71**), similar to procedure of compound **77**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO) δ 3.58 (s, 1H), 5.33 (d, 1H, *J* = 1.58 Hz), 5.89 (d, 2H, *J* = 7.4 Hz), 7.03-7.08 (m, 1H), 7.28-7.43 (m, 5H), 7.54-7.61 (m, 4H), 7.73 (d, 1H, *J* = 8.34 Hz), 8.72 (d, 1H, *J* = 8.37 Hz), 9.14 (s, 1H), 10.50 (s, 1H), 10.66 (d, 1H, *J* = 7.5 Hz); MS (ES+) 471 (M+H), m.p. > 250°C.

5.3.1.67 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (cyclopentylcarbamoyl-phenyl-methyl)-amide (Compound 139):

Compound **139** was prepared from amidation of 7-Chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 2-amino-*N*-cyclopentyl-2-phenyl acetamide (**69**), similar to procedure of compound **77**.

R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}HNMR (DMSO) δ 1.17-1.63 (m, 8H), 3.50 (t, 1H, *J* = 2.3 Hz), 3.84-3.93 (m, 1H), 5.25 (d, 2H, *J* = 2.3 Hz), 5.58 (d, 1H, *J* = 7.8 Hz), 7.16-7.28 (m, 3H), 7.34 (d, 2H, *J* = 7.35 Hz), 8.32 (d, 1H, *J* = 7.17 Hz), 8.57 (d, 1H, *J* = 7.83 Hz), 9.04 (s, 1H), 10.41 (d, 1H, *J* = 7.83 Hz); MS (ES+) 481 (M+H), m.p. 238-240°C.

5.3.1.68 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (cyclohexylcarbamoyl-phenyl-methyl)-amide (Compound 140):

Compound **140** was prepared from amidation of 7-Chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 2-amino-*N*-cyclohexyl-2-phenyl acetamide (**70**), similar to procedure of compound **77**.

R_f 0.5 (7% Acetone/$CHCl_3$); ^{1}HNMR (DMSO) δ 1.04-1.77 (m, 10H), 3.48-3.56 (m, 2H), 5.32 (d, 2H, J = 2.3 Hz), 5.67 (d, 1H, J = 7.65 Hz), 7.25-7.43 (m, 5H), 8.31 (d, 1H, J = 7.89 Hz), 8.64 (d, 1H, J = 6.18 Hz), 9.11 (s, 1H), 10.47 (d, 1H, J = 8.31 Hz); MS (ES+) 495 (M+H), m.p. > 250°C.

5.3.1.69 7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (phenyl-phenylcarbamoyl-methyl)-amide (Compound 141):

Compound **141** was prepared from amidation of 7-Chloro-6-fluoro-1-propargyl-1,8-naphthyridine-3-carboxylic acid **76c** with 2-amino-2,*N*-diphenyl acetamide (**71**), similar to procedure of compound **77**.

R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}HNMR (DMSO) δ 3.57 ((d, 1H, J = 2.38 Hz), 5.32 (d, 2H, J = 2.3 Hz), 5.87 (d, 1H, J = 7.5 Hz), 7.02-7.07 (m, 1H), 7.26-7.41 (m, 5H), 7.45-7.59 (m, 4H), 8.66 (d, 1H, J = 7.7 Hz), 9.1 (s, 1H), 10.49 (s, 1H), 10.61 (d, 1H, J = 7.5 Hz); MS (ES+) 489 (M+H), m.p. > 250°C.

5.3.1.70 6-Fluoro-4-oxo-1-prop-2-ynyl-7-pyrrolidin-1-yl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (phenyl-phenylcarbamoyl-methyl)-amide (Compound 142):

Triethylamine (1.1 g, 10mmol) and pyrrolidine (2.13 g, 11 mmol) were added to a suspension of compound **141** (4.89 g, 10 mmol) in acetonitrile (50 ml) and refluxed for 3h. The reaction mixture was cooled; the precipitate thus separated was collected by filtration, washed with acetonitrile and dried to give compound **142**. Yield 4.52 g (86.42 %). R_f 0.4 (2% Methanol/ DCM); ^{1}HNMR (DMSO) δ 1.94 (s, 4H), 3.45 (s, 1H), 3.75 (bs, 4H), 5.23 (s, 2H), 5.85 (d, 1H, J = 7.9 Hz), 7.04 (s, 1H), 7.26-7.59 (m, 9H), 7.94-7.98 (m, 1H), 8.8 (s, 1H), 10.45 (s, 1H), 10.98 (d, 1H, J = 7.4 Hz); MS (ES+) 523 (M+H), m.p. > 250°C.

5.3.2. Procedure for synthesis of 1-propargyl pyrido[2,3-c]pyridazine-3-carboxamide derivatives Series 6, Scheme 9:

5.3.2.1 2-Chloronicotinoyl chloride (Compound 143):

The suspension of 2-chloro nicotinic acid (**1a**) (5 g, 31.73 mmol) and thionyl chloride (25 ml) was heated to reflux for 2 hr at 70°C. The solution was concentrated under reduced pressure to provide the crude product. The compound was used a such for next step without purification.

5.3.2.2 3-(2-chloropyridin-3-yl)-2-diazo-3-oxopropionic acid ethyl ester (Compound 144):

2-Chloronicotionyl chloride (**143**) (3.5 g, 19.88 mmol) and ethyl diazoacetate (7.94 g, 69.60 mmol) were heated together at 55°C for 3h then left at ambient temperature for 18h. The mixture was concentrated in vacuum and the residue was chromatographed on silica gel using 30% EtOAc/ Hexane as an eluent.

R_f 0.4 (20 % EtOAc/ Hexane); ^{1}HNMR ($CDCl_3$) δ 1.17 (t, 1H, J = 7.1 Hz), 4.18 (q, 1H, J = 7.1 Hz), 7.31 (dd, 1H, J = 4.8 Hz), 7.63 (dd, 1H, J = 1.9 Hz), 8.47 (dd, 1H, J = 1.8 Hz); MS (ES+) m/z (relative intensity) 275 (M+H) (100).

5.3.2.3 Ethyl 2-hydrazono-2-(2-chloronicotinoyl) acetate (Compound 145):

Compound **144** (3.0 g, 11.82 mmol) in diisopropyl ether was treated with triphenylphosphine (3.41 g, 13.01 mmol) and stirred at ambient temperature for 18h. The resulting solid was filtered off and dried under vacuum. Crude product was chromatographed on silica gel with DCM as an eluent.

R_f 0.4 (2 % MeOH/ DCM); ^{1}HNMR ($CDCl_3$) δ 1.17 (t, 1H, J = 7.1 Hz), 4.18 (q, 1H, J = 7.1 Hz), 7.31 (dd, 1H, J = 4.9 Hz), 7.63 (dd, 1H, J = 1.1 Hz), 8.47 (d, 1H, J = 3.3 Hz).

5.3.2.4 4-Oxo-1,4-dihydrorido[2,3-c]pyridazine-3-carboxylic acid ethyl ester (Compound 146):

Compound **145** (2 g, 7.82 mmol) was heated at 80°C in a solution of methanol and water (5:1) 50 ml for 2 h. The methanol was distilled off and the resulting aqueous extracted with EtOAc. The organic was dried and evaporated. The product was purified using 20% EtOAc/ hexane.

R_f 0.5 (50% EtOAc/ Hexane); ^{1}HNMR (DMSO) δ 1.35 (t, 3H, *J* = 7.1 Hz), 4.37 (q, 2H, *J* = 7.1 Hz), 7.45-7.56 (m, 1H), 7.84 (dd, 1H, *J* = 1.8 Hz), 8.41-8.46 (m, 1H), 10.43 (bs, 1H).

5.3.2.5 4-Oxo-1-prop-2-ynyl-1,4-dihydro-pyrido[2,3-c]pyridazine-3-carboxylic acid ethyl ester (Compound 147):

To a suspension of sodium hydride (95%) (0.575 g, 22.81 mmol) in DMF (20 ml), compound **146** (2.0 g, 9.12 mmol) was added dropwise after dissolving in DMF (5 ml). The reaction mixture was heated at 60°C for 1h. Propargyl amine (1.256 g, 22.81 mmol) was added to it and again heated at 60°C for 4h. The reaction mixture was diluted with cold water. Organic layer was extracted in ethyl acetate, dried over sodium sulphate and concentrated to dryness to afford a crude product. The crude product was chromatographed on silica gel with 30 % EtOAc/ hexane as eluent.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.40-1.45 (m, 3H), 2.38 (t, 1H, *J* = 2.4 Hz), 4.47 (t, 2H, *J* = 7.1 Hz), 5.43 (d, 2H, *J* = 2.4 Hz), 7.48 (dd, 1H, *J* = 4.5 Hz), 8.68 (dd, 1H, *J* = 1.7 Hz), 8.90 (dd, 1H, *J* = 1.7 Hz); MS (ES+) m/z (relative intensity) 280 (M+H) (100).

5.3.2.6 4-Oxo-1-prop-2-ynyl-1,4-dihydro-pyrido[2,3-c]pyridazine-3-carboxylic acid (Compound 148):

A suspension of compound **147** (2g, 7.77 mmol) in a solution of dioxane/ water (1:1) (20 ml) was treated with 40% NaOH solution (10 ml) and refluxed for 6h. The solution was cooled and acidified to pH 2 with conc. HCl. Solid precipitates thus obtained were filtered and dried under vacuum to provide compound **148**.

R_f 0.3 (15% MeOH/DCM); ^{1}HNMR (DMSO) δ 3.34 (bs, 1H), 3.46 (t, 1H, *J* = 2.4 Hz), 5.45 (d, 2H, *J* = 2.3 Hz), 7.70 (dd, 1H, *J* = 4.5 Hz), 8.62 (dd, 1H, *J* = 1.8 Hz), 9.06 (dd, 1H, *J* = 1.8 Hz); MS (ES+) m/z (relative intensity) 230 (M+H) (40), 252 (M+H+Na) (100).

5.3.2.7 3-*N*-(3'-*N*'-cyclopentyl-2'-hydroxy-1'-phenylpropane-3'-carboxamide)-1-propargyl-pyrido[2,3-*c*] pyridazine-3-carboxamide (Compound 149):

To a suspension of 0.50 g (2.18 mmol) of compound **148** in DMF (20 ml), 2 ml of thionyl chloride was added dropwise at room temperature. The stirring was continued

for 4h at room temperature and reaction mixture was dried under vacuum to provide acyl chloride intermediate. The acyl chloride intermediate was diluted with dichloromethane (20 ml) and 0.812 g (3.27 mmol) of 1-*N*-cyclopentyl- 3- amino-2-hydroxy-3- phenylpropane-1-carboxamide (**39**) was added to it and stirred for 2h. The reaction mixture was left overnight, concentrated; water was added and extracted with ethylacetate. The organic layer was dried (Na_2SO_4) and concentrated to dryness to provide crude product. The crude product was purified by 2% MeOH/DCM as eluent on silica gel.

R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.16-1.71 (m, 8H), 3.93-4.00 (m, 1H), 4.17 (d, 1H, J = 3.12 Hz), 5.45 (d, 3H, J = 7.9 Hz), 5.74 (s, 1H), 6.10 (d, 1H, J = 5.5 Hz), 7.18-7.48 (m, 6H), 7.75 (dd, 1H, J = 8.1, 4.5 Hz), 8.68 (dd, 1H, J = 8.1, 1.6 Hz), 9.08 (dd, 1H, J = 4.3, 1.6 Hz), 10.14 (d, 1H, J = 8.6 Hz); MS (ES+) 460 (M+H); MS (ES+) m/z (relative intensity) 460 (M+H) (100); HPLC purity = 99.7 %.

5.3.2.8 3-*N*-(3'-*N*'-cyclohexyl-2'-hydroxy-1'-phenylpropane-3'- carboxamide)-1-propargyl-pyrido[2,3-*c*] pyridazine-3-carboxamide (Compound 150):

Compound **152** was prepared from amidation of compound 1-propargyl-pyrido[2,3-*c*] pyridazine-3-carboxylic acid (**148**) with 1-*N*-cyclohexyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**40**), similar to procedure of compound **149**.

R_f 0.3 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 0.95-1.81 (m, 10H), 2.37 (s, 1H), 3.74 (bs, 1H), 4.54 (s, 2H), 5.54 (s, 2H), 5.72 (d, 1H, J = 7.6 Hz), 6.57 (d, 1H, J = 7.4 Hz), 7.29-7.56 (m, 6H), 8.78 (d, 1H, J = 7.4 Hz), 8.97 (s, 1H), 10.81 (d, 1H, J = 7.4 Hz); MS (ES+) m/z (relative intensity) 474 (M+H) (40), 496 (M+H+Na) (100); HPLC purity = ~ 93.1 %.

5.3.2.9 3-*N*-(3'-*N*'-Phenyl-2'-hydroxy-1'-phenylpropane-3'-carboxamide)-1-propargyl-pyrido[2,3-*c*] pyridazine-3-carboxamide (Compound 151):

Compound **151** was prepared from amidation of compound 1-propargyl-pyrido[2,3-*c*] pyridazine-3-carboxylic acid (**148**) with 1-*N*-phenyl- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**41**), similar to procedure of compound **149**.

R_f 0.3 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.35 (s, 1H), 4.74 (d, 1H, J = 4.1 Hz), 5.10 (d, 1H, J = 5.8 Hz), 5.51 (d, 2H, J = 1.8 Hz), 5.80 (d, 1H, J = 4.8 Hz), 7.05-7.08 (m, 1H), 7.29-7.59 (m, 9H, 2H partially merged with $CDCl_3$ peak), 8.74 (d, 2H, J =

6.4 Hz), 8.97 (d, 1H, *J* = 2.8 Hz), 10.83 (d, 1H, *J* = 7.9 Hz); MS (ES-) m/z (relative intensity) 466 (M-H) (100); HPLC purity = 99.5 %.

5.3.2.10 3-*N*-(3'-*N*'-(2''-pyridine)-2'-hydroxy-1'-phenylpropane-3'-carboxamide)-propargyl- pyrido[2,3-*c*] pyridazine-3-carboxamide (Compound 152):

Compound **152** was prepared from amidation of compound 1-propargyl-pyrido[2,3-*c*] pyridazine-3-carboxylic acid (**148**) with 1-*N*-2'-pyridine- 3- amino-2-hydroxy-3-phenylpropane-1-carboxamide (**47**), similar to procedure of compound **149**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 2.35 (s, 1H), 3.47 (m, 1H), 5.51 (s, 2H), 5.98 (d, 1H, *J* = 8.3 Hz), 6.86-7.01 (m, 2H), 7.26-7.65 (m, 7H), 8.23 (d, 1H, *J* = 8.2 Hz), 8.78 (d, 1H, *J* = 7.4 Hz), 8.94 (s, 1H), 9.59 (s, 1H), 10.67 (d, 1H, *J* = 7.9 Hz); MS (ES+) m/z (relative intensity) 469 (M+H) (100), 491 (M+H+Na) (60); HPLC purity = 93.7 %.

5.3.2.11 3-(*N*-cyclopentyl phenyl glycinamide)-1-propargyl- pyrido[2,3-*c*] pyridazine-3-carboxamide (Compound 153):

Compound **153** was prepared from amidation of compound 1-propargyl-pyrido[2,3-*c*] pyridazine-3-carboxylic acid (**148**) with 2-amino-*N*-cyclopentyl-2-phenyl acetamide (**69**), similar to procedure of compound **149**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.25-1.84 (m, 8H), 3.44 (s, 1H), 3.94-4.02 (m, 1H), 5.47 (d, 2H, *J* = 2.1 Hz), 5.68 (d, 1H, *J* = 7.7 Hz), 7.25-7.51 (m, 5H), 7.73 (dd, 1H, *J* = 8.0, 4.4 Hz), 8.41 (d, 1H, *J* = 7.08 Hz), 8.68 (dd, 1H, *J* = 8.0, 1.7 Hz), 9.07 (dd, 1H, *J* = 4.35, 1.68 Hz), 10.31(d, 1H, *J* = 7.8 Hz); MS (ES+) m/z (relative intensity) 430 (M+H) (100); HPLC purity = 99.3 %.

5.3.2.12 3-(*N*-cyclohexyl phenyl glycinamide)-1-propargyl- pyrido[2,3-*c*] pyridazine-3-carboxamide (Compound 154):

Compound **154** was prepared from amidation of compound 1-propargyl-pyrido[2,3-*c*] pyridazine-3-carboxylic acid (**148**) with 2-amino-*N*-cyclohexyl-2-phenyl acetamide (**70**), similar to procedure of compound **149**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.06-1.79 (m, 10H), 3.43-3.49 (m, 2H), 5.46 (d, 2H, *J* = 2.2 Hz), 5.68 (d, 1H, *J* = 7.8 Hz), 7.24-7.46 (m, 5H), 7.73 (dd,

1H, J = 8.0, 4.4 Hz), 8.33 (d, 1H, J = 7.7 Hz), 8.68 (dd, 1H, J = 8.0, 1.7 Hz), 9.07 (dd, 1H, J = 4.4, 1.8 Hz), 10.31 (d, 1H, J = 7.8 Hz); MS (ES+) m/z (relative intensity) 444 (M+H) (30), 466 (M+H+Na) (100); HPLC purity = 99.7 %.

5.3.2.13 3-(*N*-phenyl phenyl glycinamide)-1-propargyl- pyrido[2,3-*c*] pyridazine-3-carboxamide (Compound 155):

Compound **155** was prepared from amidation of compound 1-propargyl-pyrido[2,3-*c*] pyridazine-3-carboxylic acid (**148**) with 2-amino-2,*N*-diphenyl acetamide (**71**), similar to procedure of compound **149**.

R_f 0.5 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 3.44 (s, 1H), 5.47 (s, 2H), 5.89 (d, 1H, J = 6.3 Hz), 7.05 (m, 1H), 7.29-7.72 (m, 10H), 8.69 (d, 1H, J = 7.3 Hz), 9.07 (s, 1H), 10.48 (m, 2H); MS (ES-) m/z (relative intensity) 436 (M-H) (100); HPLC purity = 99.6 %.

CHAPTER-6

SUMMARY & CONCLUSION

6. SUMMARY & CONCLUSION

The aim of study was to synthesize, characterize and to study the cytotoxicity of betulinic acid and naphthydrine derivatives.

1. Betulinic acid derivative Series:

To further improve the cytotoxicity and pharmacokinetic properties of betulinic acid, various modifications were carried out at different positions. By inducing heterocyclic indole group at C-2 and C-3 positions of betulinic acid, the effect of variation in hydrogen bonding potential, pKa, lipophilicity and selectivity were observed. Further changes were also carried out by making the modifications at C-20 unsaturated bond and C-28 carboxylic functional group. The compounds synthesized were tested for cytotoxicity against prostate, lung, laryngeal, pancreas, breast, colon and ovarian cancer, leukemia and lymphoma, human tumor cell lines. Compound **5** is the most active compound amongst these derivatives. Compound **5** has two times more activity on PA1 cancer line with IC_{50} of 5.8 μg/ml (betulinic acid with IC_{50} of 11.53 μg/ml). Major enhancements in the cytotoxicity was observed on SW620 and Miapaca cancer cell lines with IC_{50} of 8.4 and 6.4 μg/ml, respectively.

2. Synthesis of functionalized amino acids and novel 1,8-naphthyridine-3-carboxamide derivatives:

In our efforts to find out a potent molecule and to understand SAR in 1,8-naphthyridine derivatives, we have focused on the C-3 position and modified the C-3 carboxylic acid with different functionalized amino acids to afford 1,8-naphthyridine-3-carboxamide derivatives along with the conversion of 1,8-naphthyridine ring to pyrido[2,3-*c*]pyridazine ring system. The amide linkage may provide hydrophilic interaction while functionalized amino acids may interact with the receptors and as a consequence, it could trigger physiological response. We have reported the synthesis and *in vitro* cytotoxicity of 1,8-naphthyridine derivatives. Few of the molecules synthesized were also investigated for their potential anti-inflammatory activity. Among these derivatives, compounds **85** and **92** have showed potent cytotoxicity on PA-1 (ovary) with IC_{50} of 0.41 μM and 1.19 μM, respectively. Compound **103** has

showed IC_{50} of 0.41 μM and 0.77 μM on pancreas (Miapaca) and leukemia (K-652) cancer cell lines, respectively. Based on these studies, compounds **85** and **92** and **103** have been selected as 'Lead molecules' and further studies are under progress to determine the ADME characteristics and in vivo activity in animal models.

CHAPTER-7

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7. REFERENCES

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CHAPTER-8

LIST OF PUBLICATIONS/ PATENTS

8. List of Publications/ Patents

1. Synthesis and cytotoxic activity of heterocyclic ring-substituted betulinic acid derivatives. **Vivek Kumar**, Nidhi Rani, Pawan Aggarwal, Vinod K. Sanna, Anu T. Singh, Manu Jaggi, Narendra Joshi, Pramod K. Sharma, Raghuveer Irchhaiya, Anand C. Burman. *Bioorg Med chem. Lett*, 2008, *18*, 5058-5062.
2. Synthesis of Functionalised Amino Acid Derivatives as New Pharmacophores for Designing Anticancer Agents-**Vivek Kumar**, Mukesh M. Mudgal, Nidhi Rani, Amrita Jha, Manu Jaggi, Anu T. Singh, Vinod K. Sanna, Pramod K. Sharma, Raguveer Irchhaiya and Anand C. Burman – *J. Enz. Inhib. Med. Chem.* 2009, *24 (3)*, 769-776.
3. 1,8-Naphthyridine-3-carboxamide derivatives with anticancer and anti-inflammatory activity- **Vivek Kumar**, Manu Jaggi, Anu T. Singh, Alka Madaan, Vinod K. Sanna, Pratibha Singh, Pramod K. Sharma, Raguveer Irchhaiya and Anand C. Burman. *E. J. Med. Chem.*, 2009, *44*, 3356-3362.
4. Anticancer and Immunomodulatory Activities of Novel 1,8-Naphthyridine Derivatives- **Vivek Kumar**, Alka Madaan, Vinod K. Sanna, Manupriya Vishnoi, Narendra Joshi, Anu T. Singh, Manu Jaggi, Pramod K. Sharma, Raguveer Irchhaiya and Anand C. Burman - *J. Enz. Inhib. Med. Chem.* 2009, 1-10, iFirst.
5. Protection of phenyl glycine using BOC anhydride; Boc protection of an amino acid http://www.syntheticpages.org/pages/294
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7. Betulinic acid derivatives as Anticancer Agents: Structure activity relationship. Rama mukherjee, **Vivek Kumar**, Sanjay K. Srivastava and Anand C. Burman. *Anti-cancer Agents in Medicinal Chemistry*, 2006, *6 (3)*, 271-279.
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9. Novel Betulinic Acid Derivatives, PCT application number WO2006/085334 (published).
10. Novel 1,8-Naphthydrine derivatives as Anticancer Agents, Patent application number 3193/DEL/2005.

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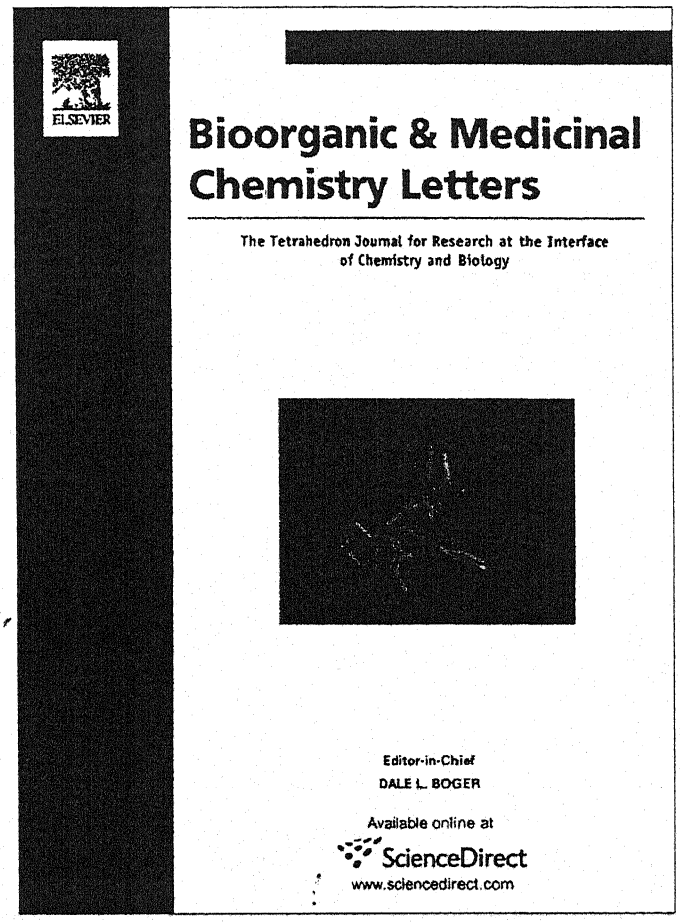

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Synthesis and cytotoxic activity of heterocyclic ring-substituted betulinic acid derivatives

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ABSTRACT

A new series of betulinic acid derivatives have been synthesized by introducing heterocyclic ring between C-2 and C-3 positions of betulinic acid. Further modifications were also carried out by reduction of C-20(29) unsaturated bond and substitution of C-28 carboxyl group by ester and amide linkage to enhance the selectivity. Compound **11** resulted in IC_{50} of 2.44, 2.5, and 2.7 μg/ml on MIAPaCa, PA-1, and SW620 cancer cell lines, respectively. Compound **38** resulted in IC_{50} of 0.67 μg/ml on MIAPaCa cell line.

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Natural products played a major role in the anticancer drug discovery. Over 60% of the anticancer drugs are of natural origin. Betulinic acid (**1**), 3β-hydroxy-lup-20(29)-en-28-oic acid, a naturally occurring pentacyclic lupane-type triterpene, is widely distributed throughout the tropics. A variety of biological properties are ascribed to betulinic acid, but betulinic acid is recognized for its anticancer and anti-HIV activities.[1–4] Previous reports revealed that betulinic acid is a melanoma-specific cytotoxic agent,[5] but recent evidence has indicated that betulinic acid also possesses a broader spectrum of cytotoxic activity against other cancer cell lines.

Various modifications of substituents at positions 2, 3, 20, and 28 of betulinic acid have been the subject matter of all research efforts to obtain potent lead compounds.[6–11] All the above mentioned reports collectively disclose a large number of betulinic acid derivatives, with a vast majority of them found to possess antitumor activity. However, due to various reasons they are not particularly good candidates, clinically as well as do not have the best of pharmacokinetic properties. A need therefore exists for novel betulinic acid derivatives, which are not only potent, but also, clinically safe and moreover, have better pharmacokinetic properties. In our efforts, we have found that in betulinic acid (**1**) heterocyclic ring-like indole, benzofuran and pyrrole at C-2 and C-3 positions, imparts the desired characteristics. Further changes were also carried out by reduction of C-20(29) unsaturated bond and substitution of C-28 carboxyl group by ester and amide linkage to enhance the selectivity (Table 1).[12]

Synthesis of betulinic acid derivatives has been described in Schemes 1–3.[13] Betulinic acid (**1**) was acetylated with acetic anhydride in presence of pyridine to afford 3-acetyloxy betulinic acid (**2**). Compound **2** upon hydrogenation with Pd/C in presence of hydrogen gas afforded 3-acetyloxy-20,29-dihydrobetulinic acid (**3**).[14] Dihydrobetulinic acid (**4**) was obtained by deacetylation of 3-*O*-acetyl 20,29-dihydrobetulinic acid (**3**) under basic conditions as shown in Scheme 1.

Both betulinic acid (**1**) and dihydrobetulinic acid (**4**) were oxidized in presence of Jones reagent to their respective betulonic acid (**5**) and 3-*O*-dihydrobetulinic acid (**6**), respectively.[15] Compounds **5** and **6** undergo Fischer indole synthesis with various arylhydrazines by loss of ammonia to afford compounds **7-19**.[16] *N*-Benzylpyrrolo substituted betulinic acid derivative (**20**) was afforded by the reaction of intermediate formed from the reaction of benzylamine with dihydrobetulinic acid (**6**), then with Michael acceptor nitroalkene.[17] Benzofuran derivatives (**21** and **22**) were synthesized from betulonic acid (**5**) and 3-*O*-dihydrobetulinic acid (**6**) with *O*-phenylhydroxylamine in presence of methanesulfonic acid as shown in Scheme 2.[18]

Ester derivatives **23–27** were synthesized by the reaction of compounds **7** and **11** with corresponding halides under basic condition. To synthesize amide derivatives (**28–37**), C-28 carboxylic group was converted to acyl chloride intermediate, which was further reacted with the corresponding amines. Compounds **28** and **29** upon hydrolysis under basic condition afforded compounds

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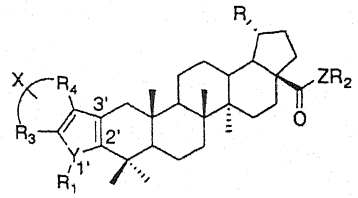
Table 1
Betulinic acid derivatives



Compound	X	Y	R	R^1	Z	R^2	R^3	R^4
7	H	N	C(=CH_2)CH_3	H	O	H		Ph
8	H	N	$CH(CH_3)_2$	H	O	H		Ph
9	H	N	C(=CH_2)CH_3	CH_3	O	H		Ph
10	H	N	$CH(CH_3)_2$	CH_3	O	H		Ph
11	5′-Cl	N	C(=CH_2)CH_3	H	O	H		Ph
12	5′-Cl	N	$CH(CH_3)_2$	H	O	H		Ph
13	5′-F	N	C(=CH_2)CH_3	H	O	H		Ph
14	5′-F	N	$CH(CH_3)_2$	H	O	H		Ph
15	7′-Cl	N	C(=CH_2)CH_3	H	O	H		Ph
16	7′-F	N	C(=CH_2)CH_3	H	O	H		Ph
17	4′-Cl, 6′-Cl	N	C(=CH_2)CH_3	H	O	H		Ph
18	5′-Cl, 7′-Cl	N	C(=CH_2)CH_3	H	O	H		Ph
19	5′-OCH_3	N	C(=CH_2)CH_3	H	O	H		Ph
20	–	N	$CH(CH_3)_2$	$C_6H_5CH_2$	O	H	H	Ph
21	H	O	C(=CH_2)CH_3	–	O	H		Ph
22	H	O	$CH(CH_3)_2$	–	O	H		Ph
23	H	N	C(=CH_2)CH_3	H	O	$CH_2C(O)OC(CH_3)_3$		Ph
24	H	N	C(=CH_2)CH_3	H	O	$CH_2CH{=}CH_2$		Ph
25	H	N	C(=CH_2)CH_3	H	O	CH_2Ph		Ph
26	5′-Cl	N	C(=CH_2)CH_3	H	O	CH_2Ph		Ph
27	H	N	C(=CH_2)CH_3	H	O	CH_2PhNO_2(4)		Ph
28	H	N	C(=CH_2)CH_3	H	NH	$CH_2CO_2CH_3$		Ph
29	5′-Cl	N	C(=CH_2)CH_3	H	NH	$CH_2CO_2CH_3$		Ph
30	5′-Cl	N	C(=CH_2)CH_3	H	NH	$CH_2C{\equiv}CH$		Ph
31	5′-Cl	N	C(=CH_2)CH_3	H	NH			Ph
32	5′-Cl	N	C(=CH_2)CH_3	H	NH			Ph
33	5′-Cl	N	C(=CH_2)CH_3	H	NH	$C_6H_4CF_3$ (4″)		Ph
34	5′-Cl	N	C(=CH_2)CH_3	H	NH	$C_6H_4OCF_3$ (4″)		Ph
35	H	N	C(=CH_2)CH_3	H	NH	CH_2Ph		Ph
36	5′-Cl	N	C(=CH_2)CH_3	H	NH			Ph
37	5′-Cl	N	C(=CH_2)CH_3	H	NH			Ph
38	H	N	C(=CH_2)CH_3	H	NH	CH_2CO_2H		Ph
39	5′-Cl	N	C(=CH_2)CH_3	H	NH	CH_2CO_2H		Ph
40	H	N	C(=CH_2)CH_3	$C_6H_5CH_2$	NH	CH_2CO_2H		Ph
41	5′-Cl	N	C(=CH_2)CH_3	$C_6H_5CH_2$	NH	CH_2CO_2H		Ph

38 and **39**. In a similar way, compounds **40** and **41** were synthesized by benzylation at *N*-1 position using sodium hydride, followed by hydrolysis as shown in Scheme 3.

Results and discussion. All the synthesized betulinic acid derivatives (**7–41**) were tested for in vitro cytotoxicity on seven tumor cell lines as well as on one non-tumorous cell line, and IC_{50} values were calculated in micromole (μg/ml).[19,20] The human tumor cell lines used in the study are ovary (PA-1), prostate (DU145), colon (SW620), breast (HBL100), pancreas (MIAPaCa2), lung (A-549), and leukemia (K562) cancers. Compounds (**7–41**) were also screened against normal mouse fibroblast (NIH3T3) to evaluate their cancer cell specificity (safety index).[19] The cytotoxicity data are summarized in Table 2.

Unsubstituted indolo betulinic acid derivative (**7**) resulted in broad spectrum of cytotoxicity with IC_{50} of 5.15, 6.01, and 6.7 μg/ml on SW620, PA-1, and MIAPaCa cancer cell lines, respectively. Reduction of C-20(29) double bond led to inactive compound **8**. Introduction of electron-donating methyl group at N-1 position in indolo betulinic acid derivative (**9**) showed selectivity toward MIAPaCa cancer cell line with IC_{50} of 5.34 μg/ml. No major affect on activity was observed (**10**) upon reduction of C-20(29) double bond.

Introduction of the electron-withdrawing halogen group in indolo ring imparted potent cytotoxicity. Compound **11** having chloro group at C-5′ position is the most potent compound of this series. Compound **11** showed IC_{50} of 2.44, 2.5, and 2.7 μg/ml on MIAPaCa, PA-1, and SW620 cancer cell lines, respectively, with safety index of ~2 on MIAPaCa. Reduction of C-20(29) double bond (**12**) caused 2- to 3-fold decrease in the activity. However, when we replaced the chloro group with fluoro at C-5′ position (**13**), major fall in activity was observed. Reduction of double bond (**14**) caused some improvement in activity on MIAPaCa and A549 cancer cell lines. On changing the position of halo group from C-5′ to C-7′ in indolo ring, chloro-substituted derivative **15** resulted in maximum

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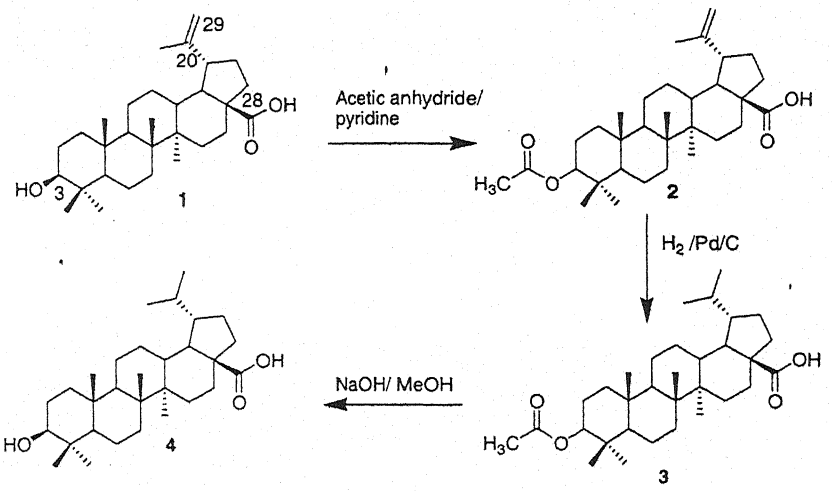
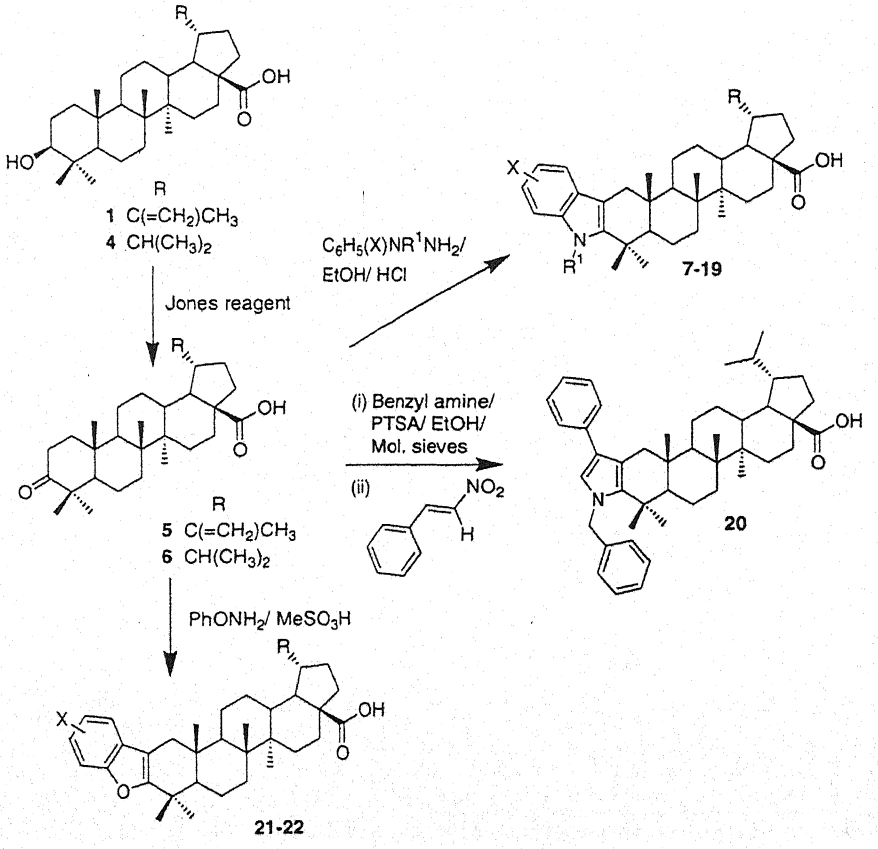


Scheme 1.



Scheme 2.

activity on SW620 cancer line with IC_{50} of 2.00 μg/ml, with safety index of ~6. Exchange of chloro with fluoro led to compound **16**, which showed potent activity with IC_{50} of <3.5 μg/ml on three cancer cell lines. Substitution of the indolo ring with dihalo electron-withdrawing group provided the selectivity toward A549 cancer cell line in compound **17**, but compound **18** was inactive. Replacing the electron-withdrawing halo group with electron donating methoxy group (**19**) did not result in substantial change in the activity.

N-Benzyl pyrrolo-substituted betulinic acid derivative (**20**) led to complete loss in the activity. Replacement of the indolo part with isosteric benzofuran led to inactive compound **21**. While reduction of C-20(29) double bond in benzofuran derivative (**22**) resulted in moderate activity on A549 cancer cell line.

As indolo-substituted betulinic acid derivatives **7** and **11** might have broad spectrum of cytotoxicity, their C-28 carboxyl group was further replaced with different ester and amide linkage. Replacement of the C-28 carboxyl group with ester groups like Boc (**23**),

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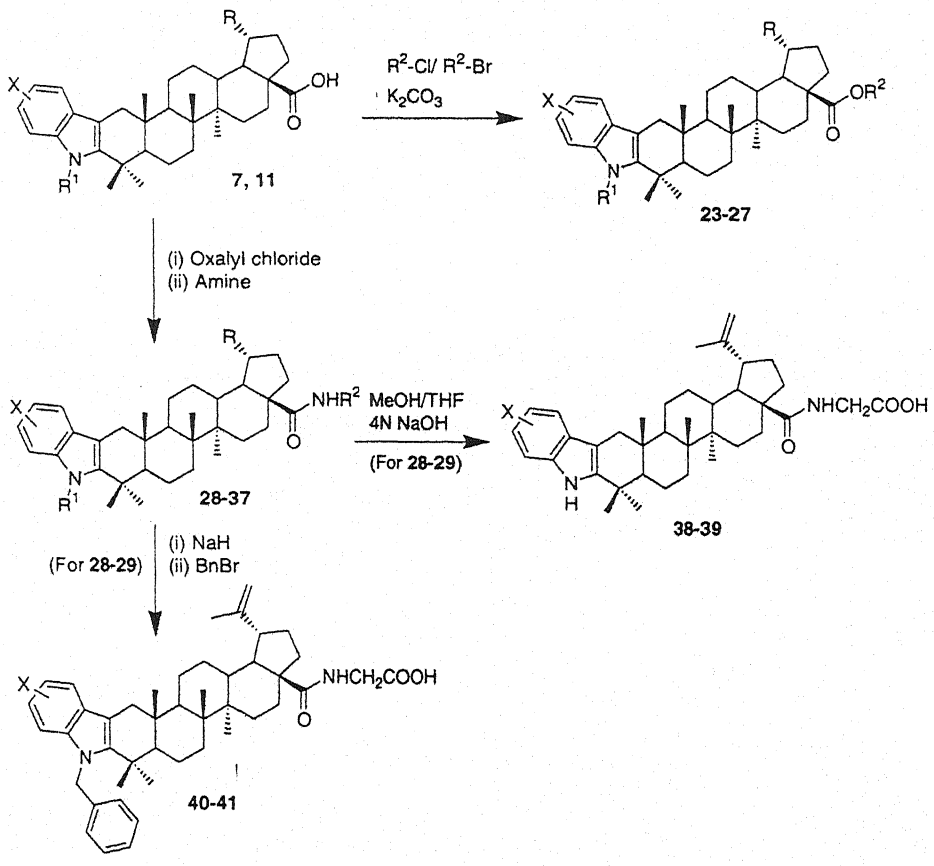

Scheme 3.

Table 2
IC_{50} values of in vitro cytotoxicity of betulinic acid (BA) derivatives

Compound	IC_{50} (μg/ml) for cell lines							
	PA-1	DU145	SW620	HBL100	MIAPaCa	A549	K562	NIH3T3
1	11.53 ± 0.8	>20	13.26 ± 0.64	5.02 ± 0.7	>20	3.00 ± 0.7	3.25 ± 1.2	4.37 ± 0.7
7	6.01 ± 0.9	8.86 ± 0.2	5.15 ± 0.7	10.3 ± 0.9	6.7 ± 0.59	7.6 ± 0.57	10.05 ± 0.61	6.9 ± 0.76
9	>20	20 ± 0.6	>20	19.5 ± 2.1	5.34 ± 0.96	6.9 ± 0.21	7.73 ± 0.17	15.12 ± 3.04
10	>20	>20	>20	14.14 ± 0.8	5.4 ± 0.43	6.5 ± 3.4	10.85 ± 1.5	>20
11	2.5 ± 0.6	4.9 ± 0.9	2.7 ± 0.2	11.75 ± 1.65	2.44 ± 0.26	7.14 ± 0.5	9.61 ± 0.78	4.6 ± 0.14
12	6.6 ± 0.9	6.5 ± 1.2	5.9 ± 0.8	>20	15.19 ± 1.06	8.8 ± 0.74	>20	19.03 ± 0.21
13	>20	>20	9.16 ± 0.8	>20	17.4 ± 2.6	17.8 ± 1.79	10.95 ± 0.93	>20
14	>20	>20	7.28 ± 0.3	>20	8.4 ± 0.76	8.89 ± 0.84	14.01 ± 0.75	14.59 ± 0.33
15	6.39 ± 0.1	11.66 ± 0.71	2.00 ± 0.3	11.8 ± 0.35	11.6 ± 0.49	8.7 ± 0.19	9.26 ± 1.6	11.8 ± 0.19
16	ND	>20	>20	3.5 ± 0.9	3.3 ± 0.2	3.0 ± 0.6	8.7 ± 0.9	2.1 ± 0.55
17	>20	>20	>20	>20	>20	5.5 ± 1.1	>20	4.10 ± 0.9
19	5.8 ± 0.9	5.75 ± 0.75	8.4 ± 0.9	12.8 ± 0.39	6.4 ± 0.44	8.2 ± 0.14	>20	7.5 ± 0.48
22	>20	>20	>20	>20	8.8 ± 0.36	7.1 ± 1.6	15.28 ± 0.18	20 ± 5.6
38	3.0 ± 0.9	7.0 ± 0.7	8.7 ± 0.1	>20	0.67 ± 0.03	3.53 ± 0.82	11.92 ± 1.37	0.68 ± 0.003
39	6.66 ± 0.43	10.32 ± 0.9	10.42 ± 0.5	16.5 ± 0.6	10.7 ± 0.63	12.4 ± 1.78	>20	11.2 ± 0.63

Cytotoxicity was determined by MTT assay, as described.[19] Data shown are IC_{50} ± SD of three independent experiments. If IC_{50} was not achieved, it was represented as greater than highest concentration tested, that is, 20 μg/ml.
ND, not done.

allyl (**24**), and benzyl groups (**25–27**) led to the complete loss of the activity. Similarly, replacement of the C-28 carboxyl group with amide groups like amino acid ester (**28** and **29**), alkyl (**30**) cycloalkyl (**31** and **32**), aryl (**33–35**), and heteroaryl (**36** and **37**) amide led to the inactive compounds.

However, when we carried out the hydrolysis of ester compound **28**, it led to potent molecule **38**, which resulted in IC_{50} of 0.67, 3.0, and 3.53 μg/ml on MIAPaCa, PA-1 and A549 cancer cell lines, respectively. While compound **39** showed moderate activity with IC_{50} of 6.66 μg/ml on PA-1 cancer cell line. Substitution of N-1 position with benzyl group along with hydrolysis in compounds **28** and **29** resulted in inactive compounds **40** and **41**.

In the present study, several derivatives have shown better cytotoxicity than betulinic acid. The halo-substituted hetrocyclic ring (indolo) at C-2 and C-3 positions in betulinic acid afforded highly potent cytotoxic compounds **11**. Substitution of N-1 posi-

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tion of indolo ring led to the loss of activity. So, N-1 position should be kept unoccupied. In most of the cases, hydrogenation of C-20(29) double bond decreased the cytotoxicity. At C-28 position, carboxylic group is essential for activity, replacement of carboxyl group with ester and amide ester leads to inactive compounds. However, hydrolysis of amide ester provided highly potent compound **38**. Compounds **11** and **38** have been selected for further studies.

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19. Derivatives of betulinic acid (**7–41**) were screened for cytotoxic activity at the highest soluble concentration of 20 μg/ml and at four lower concentrations on seven human tumor cell lines and one non-tumorous cell line. Briefly, cells were collected from 70–80% confluent adherent cultures by trypsinization (0.25% trypsin and 0.02% EDTA) and seeded in 96-well plates at a density of 5000 cells/well, except K562 which was seeded at15,000 cells/well in cell culture medium (DMEM) for 24 h in a CO_2 incubator. The test substance was dissolved in DMSO (Merck, India), and further dilutions were made in cell culture medium such that the final DMSO concentration in the well even at the highest concentration is less than 1%. After 24 h, the cells were incubated with the above-mentioned test substance to obtain drug concentrations in the range of 0.5–20 μg/mL. After 72 h of incubation in a CO_2 incubator, cytotoxicity was measured by the tetrazolium-based MTT assay adapted from previously published methods.[20] Briefly, 25 μL of MTT (5 mg/ml, Sigma, USA) was added to each well of the 96-well plate, and the plate was incubated at 37 °C for 3 h. MTT was converted to greenish-brown colored formazan by mitochondrial dehydrogenase enzyme present in viable cells. For adherent cells, the medium in the wells was gently pipetted out and replaced with 150 μL of DMSO and kept with gentle shaking for 15 min to dissolve formazan crystals. For suspension cultures, formazan was dissolved by direct addition of 50 μL of sodium dodecyl sulfate (SDS) acidified with 1N HCl, added to the wells followed by incubation for 1 h and the contents were mixed using a pipetman. The optical density (O.D.) in the wells was measured at 540 nm (for adherent cells) or 570 nm (for suspension cells) using a multi-well spectrophotometer. Percentage cytotoxicity was calculated using the formula given below %Cytotoxicity = $1 - (X/R_1) * 100$ where X = O.D. of wells containing the test substance and R_1 = O.D. of control wells. Each experiment was repeated thrice and IC_{50} values (half-maximal cytotoxicity) were calculated by employing non-linear regression analysis using Prism® software.
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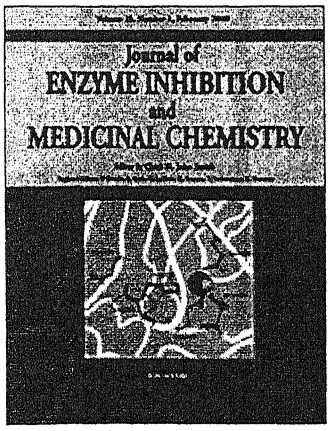

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Synthesis of functionalized amino acid derivatives as new pharmacophores for designing anticancer agents

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Synthesis of functionalized amino acid derivatives as new pharmacophores for designing anticancer agents

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Abstract

A new series of functionalized amino acid derivatives *N*-substituted 1-*N*-(tert-butoxycarbonyl)-2,2-dimethyl-4-phenyl-5-oxazolidine carboxamide (**1-17**) and 1-*N*-substituted-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide (**18-34**) were synthesized and evaluated for their *in vitro* cytotoxicity against human cancer cell lines. Compound **6** has shown interesting cytotoxicity ($IC_{50} = 5.67\ \mu m$) in ovarian cancer, while compound **10** exhibited promising cytotoxicity in ovarian ($IC_{50} = 6.1\ \mu m$) and oral ($IC_{50} = 4.17\ \mu m$) cancers. These compounds could be of use in designing new anti-cancer agents.

Keywords: *Anticancer, functionalized amino acid, carboxamide*

Introduction

Cancer is a disease of worldwide importance. According to World Health Organization (WHO) report, cancer is causing 7 million deaths every year or 12.5% of deaths worldwide [1,2]. Paclitaxel, a biggest-selling single anticancer drug, was discovered at Research Triangle Institute (RTI), USA in 1967 and brought to the market by BMS in 1993 as Taxol® [3]. During last one decade, a number of small organic molecules such as Imatinib (Gleevec), Gefitinib (Irresa), Erlotinib (Tarceva) and Canertinib have been discovered and reached to the market [4]. However, despite major breakthroughs in different areas of drug discovery, the successful treatment of the cancer still remains a significant challenge in the tewenty first century. The search for newer and safer anticancer agent associated with broader spectrum cytotoxicity is needed.

Designing of new chemical entities as anticancer agents, require simulation of a suitable bioactive pharmacophore. The pharmacophore should not only require potent but must also be safer on normal cell lines than tumor cells. Such types of information are, generally, not projected in publications. Both of the well known anticancer molecules Paclitaxel and Docetaxel have amino acids side chain, based on which we have synthesized these amino acids and tested them separately for their anticancer activity and obtained a few potent molecules which can be further used as a new anticancer pharmacophore or as side chains. The synthesis, *in vitro* cytotoxicity and their structure-activity relationship are presented here.

Materials and methods

All the solvents and reagents were purchased from different companies such as Aldrich, Lancaster, Across

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& Rankem and were used as supplied. All TLC data (R_f values) were determined on aluminum sheets coated with silica gel 60 F_{254} (Merck). Visualization was achieved with UV light and iodine vapor. Column chromatography was performed using silica gel (100–200 mesh). Proton Magnetic Resonance (PMR) spectra were recorded on a Bruker 300 MHz instrument using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Micromass Quattro Micro™ instrument. The purity of the synthesized compounds was determined on a Shimadzu HPLC LC-2010 C HT instrument using gradient system.

Chemistry

Synthesis of functionalized amino acid derivatives (**1-34**) is shown in Scheme 1. Coupling of *4S,5R*-1-*N*-(tert-butoxycarbonyl)-2,2-dimethyl-4-phenyl-5-oxazolidine carboxylic acid (**I**) with appropriate amines has been carried out to afford the respective *N*-substituted-1-*N*-(tert-butoxycarbonyl)-2,2-dimethyl-4-phenyl-5-oxazolidine carboxamide (**1-17**). The coupling reactions were performed by using either *N,N'*,-dicyclohexyl carbodiimide (DCC) and dimethylaminopyridine (DMAP) or *N*-ethyl-*N'*-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) in DCM or DMF, which were used as solvent. The oxazolidine ring of compounds **1-17** was separately opened with 50% TFA/DCM to afford the corresponding 1-*N*-substituted-3-amino-2-hydroxy-3-phenylpropane-1-carboxamides (**18-34**). The functionalized amino acid derivatives (**1-34**) are listed in Table I.

5-N-*Isopropyl-3*-N'-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(1)***. Isopropyl amine (0.92 g, 15.5 mmol) was added, to a stirred solution of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid (5 g, 15.5 mmol) in dichloromethane (100 mL). The resulting solution was placed in ice bath for 15 min. at 0°C. To this reaction mixture 1-hydroxybezotriazole hydrate (HOBt, 2.10 g, 15.5 mmol) and *N*-methylmorpholine (NMM, 1.57 g, 15.5 mmol) were added. After stirring for 30 min at 0°C, *N*-ethyl-*N'*-3-dimethylaminopropyl carbodiimide hydrochloride (EDCI, 2.9 g, 15.1 mmol) was added and the reaction mixture was maintained at 0°C for 3 h, then stirred for 5 h at rt and left overnight. Water (100 mL) was added to reaction mixture and extracted with dichloromethane (100 mL). The combined organic layer was dried over Na_2SO_4 and evaporated to afford the crude residue. The crude product was purified by column chromatography using dichloromethane/methanol as eluent, to provide the pure compound **1**.

R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.16-1.30 (m, 15H), 1.70 (s, 3H), 1.78 (s, 3H), 4.06-4.17 (m, 1H), 4.33 (d, 1H, J = 5.6 Hz), 5.09 (bs, 1H), 6.33 (bs, 1H), 7.24-7.34 (m, 5H); MS (ES +) m/z (relative intensity) 363 (M + H) (10), 385 (M + H + Na) (100); HPLC purity = 95.7%.

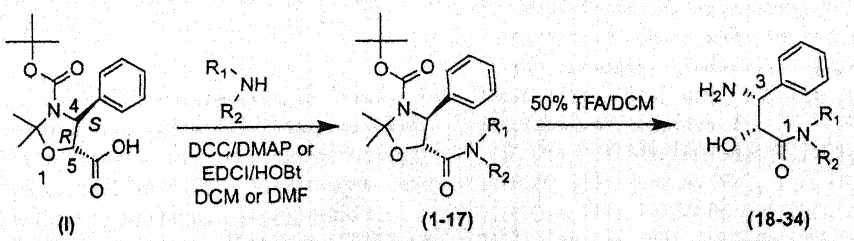
5-N-*Cyclopropyl-3*-N'-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(2)***. Compound **2** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with cyclopropylamine, similar to procedure of compound **1**.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.54-0.56 (m, 2H), 0.80-0.82 (m, 2H), 1.14 (bs, 9H), 1.68 (s, 3H), 1.75 (s, 3H), 2.73-2.76 (m, 1H), 4.32 (d, 1H, J = 5.8 Hz), 5.06 (bs, 1H), 6.57 (bs, 1H), 7.26-7.42 (m, 5H); MS (ES +) m/z (relative intensity) 383 (M + H + Na) (100); HPLC purity = 99.7%.

5-N-*Cyclopentyl-3*-N'-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(3)***. Compound **3** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with cyclopentyl amine, similar to procedure of compound **1**.

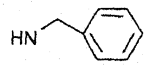
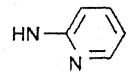
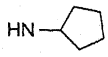
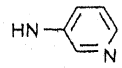
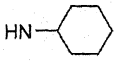
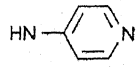
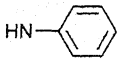
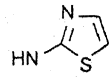
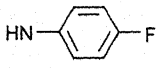
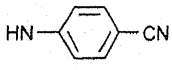
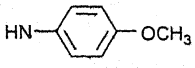
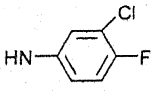
R_f 0.6 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.15 (bs, 9H), 1.38-1.46 (m, 1H), 1.63-1.69 (m, 8H), 1.77 (s, 3H), 1.96-2.0 (m, 2H), 4.18 - 4.28 (m, 1H), 4.33 (d, 1H, J = 5.5 Hz), 5.11 (bs, 1H), 6.43 (d, 1H, J = 6.9 Hz), 7.34-7.24 (m, 5H); MS (ES +) m/z (relative intensity) 389 (M + H) (10), 411 (M + H + Na) (100); HPLC purity = 99.5%.

5-N-*Cyclohexyl-3*-N'-tert-*butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide* ***(4)***. Compound **4** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carbo-



Scheme 1. Synthesis of tested compounds.

Table I. Functionalized amino acid derivatives (1-34).

Compound No.	R_1R_2N	Compound No.	R_1R_2N
1, 18		10, 27	
2, 19		11, 28	
3, 20		12, 29	
4, 21		13, 30	
5, 22		14, 31	
6, 23		15, 32	
7, 24		16, 33	
8, 25		17, 34	
9, 26			

xylic acid with cyclohexyl amine, similar to procedure of compound **1**.

R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.04-1.27 (m, 11H), 1.31-1.44 (m, 3H), 1.60-1.82 (m, 9H), 1.88-1.95 (m, 2H), 3.78-3.81 (m, 1H), 4.32 (d, 1H, J = 5.5 Hz), 5.09 (bs, 1H), 6.41 (d, 1H, J = 7.9 Hz), 7.22-7.36 (m, 5H); MS (ES +) m/z (relative intensity) 403 (M + H) (30), 425 (M + H + Na) (100); HPLC purity = 99.3%.

5-N-Phenyl-3-N′-tert-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(5)***. Compound **5** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with aniline, similar to procedure of compound **1**.

R_f 0.4 (20% EtOAc/Hexane); ^{1}HNMR ($CDCl_3$) δ 1.08 (bs, 9H), 1.72 (s, 3H), 1.76 (s, 3H), 4.44 (d, 1H, J = 5.6 Hz), 5.12 (bs, 1H), 7.07 (t, 1H, J = 7.2 Hz), 7.18-7.30 (m, 7H), 7.49-7.52 (m, 2H), 8.22 (bs, 1H); MS (ES +) m/z (relative intensity) 419 (M + H + Na) (100); HPLC purity = 93.8%.

5-N-(4′-Fluoro) phenyl-3-N′-tert-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(6)***. Compound **6** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 4-fluoro aniline, similar to procedure of compound **1**.

R_f 0.3 (DCM); ^{1}HNMR ($CDCl_3$) δ 1.16 (bs, 9H), 1.78 (s, 3H), 1.82 (s, 3H), 4.51 (d, 1H, J = 5.9 Hz), 5.16 (bs, 1H), 7.01-7.06 (m, 2H), 7.26-7.37 (m, 5H), 7.51-7.56 (m, 2H), 8.28 (bs, 1H); MS (ES +) m/z (relative intensity) 437 (M + H + Na) (100).

5-N-(4'-cyano) phenyl-3-N'-tert-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(7)***. Compound 7 was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 4-cyano aniline, similar to procedure of compound **1**.

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.14-1.17 (m, 9H), 1.78-1.83 (m, 6H), 4.52 (d, 1H, J = 6.0 Hz), 5.15 (bs, 1H), 7.25-7.37 (m, 5H), 7.63-7.73 (m, 5H, 8.45 (bs, 1H); MS (ES-) m/z (relative intensity) 420 (M-H) (100); HPLC purity = 98.3%.

5-N-(4'-Methoxy) phenyl-3-N'-tert-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(8)***. Compound 8 was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 4-methoxy aniline, similar to procedure of compound **1**.

R_f 0.2 (DCM); ^{1}HNMR ($CDCl_3$) δ 1.09 (bs, 9H), 1.71 (s, 3H), 1.75 (s, 3H), 3.78 (s, 3H), 4.43 (d, 1H, J = 5.7 Hz), 5.11 (bs, 1H), 6.79-6.82 (m, 2H), 7.18-7.32 (m, 5H), 7.39-7.42 (m, 2H), 8.11 (bs, 1H); MS (ES +) m/z (relative intensity) 427 (M + H) (10), 449 (M + H + Na) (100); HPLC purity = 95.9%.

5-N-(3'-Chloro-4'-fluoro) phenyl-3-N'-tert-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(9)***. Compound 9 was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 3-chloro-4-fluoro aniline, similar to procedure of compound **1**.

R_f 0.5 (DCM); ^{1}HNMR ($CDCl_3$) δ 1.09 (bs, 9H), 1.70 (s, 3H), 1.74 (s, 3H), 4.42 (d, 1H, J = 5.9 Hz), 5.19 (bs, 1H), 7.03 (t, 1H, J = 8.7 Hz), 7.18-7.30 (m, 6H), 7.70-7.72 (m, 1H), 8.20 (bs, 1H); MS (ES +) m/z (relative intensity) 449 (M + H) (5), 471 (M + H + Na) (100); HPLC purity = 96.4%.

5-N-Benzyl-3-N'-tert-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(10)***. Compound 10 was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with benzylamine, similar to procedure of compound **1**.

R_f 0.5 (15% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.14 (bs, 9H), 1.66 (s, 3H), 1.75 (s, 3H), 4.41-4.58 (m, 3H), 5.09 (bs, 1H), 6.84 (bs, 1H), 7.24-7.35 (m, 10H); MS (ES +) m/z (relative intensity) 433 (M + H + Na) (100); HPLC purity = 99.7%.

5-N-2'-Pyridine-3-N'-tert-butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(11)***. Compound 11 was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 2-amino pyridine, similar to procedure of compound **1**.

R_f 0.6 (2% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.14 (bs, 9H), 1.79 (s, 3H), 1.82 (s, 3H), 4.51 (d, 1H, J = 5.9 Hz), 5.16 (bs, 1H), 7.06-7.1 (m, 1H), 7.26-7.46 (m, 5H), 7.7-7.75 (m, 1H), 8.24 (d, 1H, J = 8.2 Hz), 8.31-8.32 (m, 1H), 8.91 (bs, 1H); MS (ES +) m/z (relative intensity) 398 (M + H) (10), 420 (M + H + Na) (100); HPLC purity = 95.5%.

5-N-3'-Pyridine-3-N'-tert-butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(12)***. Compound 12 was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 3-amino pyridine, similar to procedure of compound **1**.

R_f 0.7 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.16 (bs, 9H), 1.70 (s, 3H), 1.76 (s, 3H), 4.53 (d, 1H, J = 6.0 Hz), 5.17 (bs, 1H), 7.26-7.46 (m, 6H), 8.23 (d, 1H, J = 8.0 Hz), 8.36-8.4 (m, 2H), 8.64 (bs, 1H); MS (ES +) m/z (relative intensity) 398 (M + H) (30), 420 (M + H + Na) (100); HPLC purity = 95.8%.

5-N-4'-Pyridine-3-N'-tert-butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(13)***. 4-Amino pyridine (1.46 g, 15.5 mmol) and 4-dimethylamino-pyridine (DMAP, 1.90 g, 15.5 mmol) were added to a stirred solution of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid (5 g, 15.5 mmol) in dichloromethane (100 mL). The reaction mixture was placed in ice bath and after 30 min at 0°C, to this *N,N'*-dicyclohexyl carbodiimide (DCC, 3.21 g, 15.5 mmol) was added under nitrogen condition. The reaction mixture was further stirred for 5 h at rt and left overnight. Water (100 mL) was added to reaction mixture and extracted with dichloromethane (100 mL). The combined organic layer was dried over Na_2SO_4 and evaporated to afford the crude residue. The crude product was purified by column chromatography using dichloromethane/methanol as eluent. In several cases, solid was appeared during the addition of water in the reaction mixture. It was filtered, washed with water, dried and purified, as described above, to provide the pure compound.

R_f 0.7 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.17 (bs, 9H), 1.78 (s, 3H), 1.83 (s, 3H), 4.51 (d, 1H, J = 6.0 Hz), 5.16 (bs, 1H), 7.26-7.38 (m, 5H), 7.52-7.54 (m, 2H), 8.41 (bs, 1H), 8.53-8.55 (m, 2H); MS (ES +) m/z (relative intensity) 398 (M + H) (100), 420 (M + H + Na) (10); HPLC purity = 92.8%.

5-N-2'-Thiazole-3-N'-tert-butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(14)***. Compound 14 was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with 2-amino thiazole, similar to procedure of compound **1**.

R_f 0.6 (2% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.16 (bs, 9H), 1.75 (s, 3H), 1.81 (s, 3H), 4.60 (d, 1H, J = 5.9 Hz), 5.19 (bs, 1H), 7.03 (d, 1H, J = 3.5 Hz), 7.27-7.38 (m, 5H), 7.49 (d, 1H, J = 3.5 Hz), 9.92 (bs, 1H); MS (ES +) m/z (relative intensity) 404 (M + H) (5), 426 (M + H + Na) (100); HPLC purity = 98.3%.

5-N-Piperidine-3-N′-tert-butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(15)***. Compound 15 was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with piperidine, similar to procedure of compound 1.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.13 (bs, 9H), 1.54-1.63 (m, 9H), 1.76 (s, 3H), 3.25-3.38 (m, 2H), 3.48-3.54 (m, 1H), 3.74-3.78 (m, 1H), 4.54 (d, 1H, J = 5.0 Hz), 5.53 (bs, 1H), 7.24-7.36 (m, 5H); MS (ES +) m/z (relative intensity) 389 (M + H) (10), 411 (M + H + Na) (100); HPLC purity = 99%.

5-N-Morpholine-3-N′-tert-butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(16)***. Compound **16** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with morpholine, similar to procedure of compound 1.

R_f 0.6 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.07-1.37 (m, 9H), 1.51 (s, 3H), 1.67 (s, 3H), 3.39-3.52 (m, 8H), 4.76 (d, 1H, J = 4.6 Hz), 5.32 (bs, 1H), 7.26-7.37 (m, 5H); MS (ES +) m/z (relative intensity) 391 (M + H) (5), 413 (M + H + Na) (100); HPLC purity = 99.6%.

5-N-Pyrrolodine-3-N′-tert-butoxycarbonyl-2, 2-dimethyl-4-phenyl oxazolidine-5-carboxamide ***(17)***. Compound **17** was prepared from amidation of *N*-(tert-butoxycarbonyl)-3,3-dimethyl-4-phenyl-oxazolidine-5-carboxylic acid with pyrrolidine, similar to procedure of compound 13.

R_f 0.5 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.11 (bs, 9H), 1.62 (s, 3H), 1.66 (s, 3H), 1.76-1.91 (m, 4H), 3.19-3.21 (m, 1H), 3.46-3.5 (m, 2H), 3.62-3.64 (m, 1H), 4.44 (d, 1H, J = 6.0 Hz), 5.41 (bs, 1H), 7.24-7.33 (m, 5H); MS (ES +) m/z (relative intensity) 397 (M + H + Na) (100), HPLC purity = 94.6%.

1-N-Isopropyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(18)***. To 5-*N*-Isopropyl-3-*N′*-*tert*-butoxycarbonyl-2,2-dimethyl-4-phenyl oxazolidine-5-carboxamide (1, 5 g, 13.79 mmol), 50% TFA/DCM (50 mL) was added at 0°C. Reaction mixture was stirred for 4 h at rt and then left overnight. Aqueous $NaHCO_3$ saturated solution was then added till neutralization. DCM layer was separated, dried over Na_2SO_4 and evaporated to afford the crude product. The product was further purified by column chromatography using 2% MeOH/DCM as eluent.

R_f 0.2 (15% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.02-1.18 (m, 6H), 2.20 (bs, 3H), 3.99-4.1 (m, 2H), 4.57 (d, 1H, J = 2.7 Hz), 6.62 (d, 1H, J = 7.3 Hz), 7.18-7.64 (m, 5H); MS (ES +) m/z (relative intensity) 223 (M + H) (100), 245 (M + H + Na) (90), HPLC purity = 80.7%.

Compounds **19-34** were prepared in the similar way to compound **18**.

1-N-Cyclopropyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(19)***. R_f 0.2 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 0.47-0.50 (m, 2H), 0.75-0.78 (m, 2H), 1.98 (bs, 3H), 2.68-2.75 (m, 1H), 4.03 (d, 1H, J = 2.8 Hz), 4.58 (d, 1H, J = 2.8 Hz), 6.9 (bs, 1H), 7.28-7.45 (m, 5H); MS (ES +) m/z (relative intensity) 221 (M + H) (20), 243 (M + H + Na) (100), HPLC purity = 94.6%.

1-N-Cyclopentyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(20)***. R_f 0.3 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.25-1.39 (m, 2H), 1.59-1.63 (m, 3H), 1.91-2.0 (m, 3H), 4.03 (d, 1H, J = 2.8 Hz), 4.14-4.23 (m, 1H), 4.57 (d, 1H, J = 2.8 Hz), 6.74 (bs, 1H), 7.26-7.41 (m, 5H); MS (ES +) m/z (relative intensity) 249 (M + H) (30), 271 (M + H + Na) (100), HPLC purity = ~ 100%.

1-N-Cyclohexyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(21)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.05-1.42 (m, 5H), 1.59-1.84 (m, 5H), 2.32 (bs, 2H), 3.76-3.79 (m, 1H), 4.0 (s, 1H), 4.53 (s, 1H), 6.67 (d, 1H, J = 7.1 Hz), 7.26-7.40 (m, 5H); MS (ES +) m/z (relative intensity) 263 (M + H) (60), 285 (M + H + Na) (100), HPLC purity = 94.7%.

1-N-Phenyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(22)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.90 (bs, 2H), 4.08 (s, 1H), 4.20 (s, 1H), 5.8 (bs, 1H), 7.01-7.06 (m, 1H), 7.17-7.19 (m, 1H), 7.20-7.30 (m, 4H), 7.39 (d, 2H, J = 7.4 Hz), 7.64 (d, 2H, J = 7.9 Hz), 9.63 (bs, 1H); MS (ES +) m/z (relative intensity) 257 (M + H) (5), 279 (M + H + Na) (100), HPLC purity = ~ 100%.

1-N-(4′-Fluoro) phenyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(23)***. R_f 0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.90 (bs, 2H), 4.07 (s, 1H), 4.20 (s, 1H), 5.75 (bs, 1H), 7.09-7.49 (m, 7H), 7.64-7.69 (m, 2H), 9.72 (bs, 1H); MS (ES +) m/z (relative intensity) 275 (M + H) (25), 297 (M + H + Na) (100), HPLC purity = 95%.

1-N-(4'-Cyano) phenyl-3-amino-2-hydroxy-3-phenyl-propane-1-carboxamide ***(24)***. R_f 0.2 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 4.13 (d, 1H, J = 3.9 Hz), 4.22 (d, 1H, J = 3.9 Hz), 7.18-7.40 (m, 5H), 7.73 (d, 2H, J = 8.6 Hz), 7.85 (d, 2H, J = 8.6 Hz); MS (ES +) m/z (relative intensity) 282 (M + H) (40), 304 (M + H + Na) (70), HPLC purity = 92.3%.

1-N-(4'-Methoxy) phenyl-3-amino-2-hydroxy-3-phenyl-propane-1-carboxamide ***(25)***. R_f 0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 1.88 (bs, 2H), 3.71 (s, 3H), 4.04 (d, 1H, J = 3.0 Hz), 4.19 (s, 1H), 5.7 (bs, 1H), 6.86 (d, 2H, J = 8.7 Hz), 7.17-7.40 (m, 5H), 7.52-7.55 (m, 2H), 9.51 (bs, 1H); MS (ES +) m/z (relative intensity) 287 (M + H) (30), 309 (M + H + Na) (100), HPLC purity = 94.2%.

1-N-(3'-Chloro-4'-fluoro) phenyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(26)***. R_f 0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 4.07 (d, 1H, J = 3.6 Hz), 4.19 (d, 1H, J = 3.6 Hz), 7.17-7.39 (m, 6H), 7.58-7.63 (m, 1H), 7.99 (dd, 1H, J = 2.5, 6.9 Hz); MS (ES +) m/z (relative intensity) 309 (M + H) (40), 331 (M + H + Na) (100), HPLC purity = 90.8%.

1-N-Benzyl-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(27)***. R_f 0.5 (15% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 8.23 (bs, 1H), 7.37-7.15 (m, 10H), 5.54 (bs, 1H), 4.27 (d, 2H, J = 6.0 Hz), 4.14 (d, 1H, J = 2.9 Hz), 3.99 (s, 1H), 1.81 (bs, 2H); MS (ES +) m/z (relative intensity) 271 (M + H) (5), 293 (M + H + Na) (100), HPLC purity = 98.7%.

1-N-2'-Pyridine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(28)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 4.31 (d, 1H, J = 1.7 Hz), 4.71 (s, 1H), 6.88-6.92 (m, 1H), 7.26-7.47 (m, 6H), 7.65-7.7 (m, 1H), 8.27 (d, 1H, J = 8.3 Hz), 9.83 (bs, 1H); MS (ES +) m/z (relative intensity) 258 (M + H) (5), 280 (M + H + Na) (100), HPLC purity = 99.2%.

1-N-3'-Pyridine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(29)***. R_f 0.2 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 4.26 (s, 1H), 4.72 (s, 1H), 7.28-7.44 (m, 6H), 8.20 (d, 1H, J = 8.4 Hz), 8.35 (d, 1H, J = 4.5 Hz), 8.61 (s, 1H), 9.16 (bs, 1H); MS (ES +) m/z (relative intensity) 258 (M + H) (80), 280 (M + H + Na) (100), HPLC purity = ~ 100%.

1-N-4'-Pyridine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(30)***. R_f 0.5 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 4.16 (s, 1H, J = 4.2 Hz), 4.24 (d, 1H, J = 4.2 Hz), 7.19-7.40 (m, 5H), 7.64 (d, 2H, J = 6.2 Hz), 8.39 (d, 2H, J = 6.2 Hz); MS (ES +) m/z (relative intensity) 258 (M + H) (15), 280 (M + H + Na) (35).

1-N-2'-Thiazole-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(31)***. R_f 0.3 (5% MeOH/DCM) ^{1}HNMR ($CDCl_3$) δ 1.25 (s, 2H), 4.37 (d, 1H, J = 1.8 Hz), 4.71 (d, 1H, J = 1.8 Hz), 6.97 (d, 1H, J = 3.5 Hz), 7.31-7.43 (m, 6H); MS (ES +) m/z (relative intensity) 264 (M + H) (100); HPLC purity = 96.8%.

1-N-Piperidine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(32)***. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.19-1.25 (m, 1H), 1.38-1.5 (m, 5H), 2.48 (bs, 3H), 2.86-2.90 (m, 1H), 3.20-3.37 (m, 2H), 3.67-3.71 (m, 1H), 4.07 (d, 1H, J = 4.5 Hz), 4.44 (d, 1H, J = 4.5 Hz), 7.25-7.42 (m, 5H); MS (ES +) m/z (relative intensity) 249 (M + H) (50), 271 (M + H + Na) (100), HPLC purity = 94%.

1-N-Morpholine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(33)***. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 2.29 (bs, 3H), 2.80-2.85 (m, 1H), 2.98-3.03 (m, 1H), 3.20-3.24 (m, 1H), 3.30-3.63 (m, 5H), 4.12 (d, 1H, J = 5.9 Hz), 4.35 (d, 1H, J = 5.9 Hz), 7.26-7.42 (m, 5H); MS (ES +) m/z (relative intensity) 251 (M^+H) (50), 273 (M + H + Na) (100), HPLC purity = 98.8%.

1-N-Pyrrolodine-3-amino-2-hydroxy-3-phenylpropane-1-carboxamide ***(34)***. R_f 0.2 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 1.52-1.77 (m, 4H), 2.31 (bs, 3H), 2.52-2.55 (m, 1H), 3.22-3.28 (m, 2H), 3.33-3.37 (m, 1H), 4.12-4.18 (m, 2H), 7.25-7.35 (m, 3H), 7.40−7.43 (m, 2H); MS (ES +) m/z (relative intensity) 235 (M + H) (50), 257 (M + H + Na) (100), HPLC purity = 97.8%.

Cytotoxicity

Various concentrations of functionalized amino acid derivatives (**1-34**) were screened for their cytotoxic activity *in vitro* on nine different human cancer cell lines tumor and one normal cell line. Briefly, a three day MTT *in vitro* cytotoxicity assay was performed, which is based on the principle of uptake of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), a tetrazolium salt, by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically [5]. MTT was dissolved in phosphate buffered saline with a pH of 7.4 to obtain an MTT concentration of 5 mg/mL; the resulting mixture was filtered through a 0.22-micron filter to sterilize and remove a small amount of insoluble residue. The cells were seeded in 96-well culture plates at a density of 5000–10,00 cells/well and incubated with various concentrations of functionalized amino acid derivatives

Table II. *In vitro* cytotoxicity data of functionalized amino acid derivatives (1-34).

No.	IC_{50} (μM)			
	PA-1 (Ovary)	DU-145 (Prostate)	KB (Oral)	NIH3T3 (Normal fibroblast)
1	41.27	NA	45.70	NA
2	NA	NA	46.73	NA
3	NA	NA	24.89	NA
4	22.56	62.9	NA	74.5
5	9.41	64.41	NA	NA
6	5.67	27.75	12.6	86.6
7	85.17	NA	8.31	NA
8	13.13	87.72	16.12	NA
9	24.14	NA	NA	NA
10	6.1	24.34	4.17	26.2
11	89.32	NA	49.33	NA
12	37.46	69.59	NA	NA
13	NA	34.15	54.88	NA
15	57.32	NA	NA	NA
16	81.04	NA	47.88	NA
17	NA	NA	95.16	NA
21	NA	NA	22.02	NA
23	NA	NA	50.71	NA
24	69.53	25.9	12.5	NA
25	NA	NA	36.01	NA
26	56.9	84.06	36	NA
27	NA	NA	35.47	NA
29	89.54	NA	98.68	NA
30	NA	NA	37.82	NA
31	NA	NA	47.73	NA
32	NA	NA	96	NA
33	NA	NA	71.14	NA

Cytotoxicity was assessed by MTT assay as described in methods. The data shown represents the IC_{50} values obtained from the single independent experiment done in triplicates. NA represent an IC_{50} value > 100 μM.

at 37°C (1-34) in a CO_2 incubator for 72 h. Control cells, treated with the appropriate vehicle were similarly incubated. The assay was terminated after 72 h by adding 25 μl of MTT to each well, then incubating for three hours, and finally adding 50 μL of 10% SDS-0.01 N HCl to each well to lyse the cells and dissolve formazan. After incubating for one hour, the plate was read spectrophotometrically at 540 nm and percentage inhibition of cell growth was calculated using the following formula: Cytotoxicity percentage = 100 × [1-(X/R_1)], where X = (absorbance of treated sample at 540 nm) R_1 = absorbance of control sample at 540 nm.

Results and discussion

Functionalized amino acid derivatives (1-34) were screened for their *in vitro* cytotoxicity on tumor as well as a non-tumorous cell lines and IC_{50} values were determined in micro molar (μM) concentrations. The human tumor cell lines used in the screening were ovary (PA-1), prostate (DU-145), oral (KB), colon (SW620), breast (HBL100), lung (A-549), pancreas (MIAPaCa2), leukemia (K562) and endotheial (ECV304) cancer cell lines. All the functionalized amino acid derivatives (1-34) and assay standard doxorubicin HCl (data not shown) were also tested against normal mouse fibroblast (NIH3T3) cell line to evaluate their tumor cell specificity (safety index). The cytotoxicity data is summarized in Table II. The compounds, which did not show cytotoxicity, are not listed in Table II. Structure activity relationship (SAR) of these derivatives has been described below. In the present discussion, compounds having IC_{50} < 10, 10–20 and >20 μM have been designated as high, moderate and low cytotoxic derivatives, respectively.

All the *N*-alkyl oxazolindine-5-carboxamide derivatives (1-4) showed low cytotoxicity, however, *N*-cyclohexyl-oxazolindine-5-carboxamide (4) was found slightly better than its *N*-isopropyl (1), *N*-cyclopropyl (2), *N*-cyclopentyl and (3) analogues. The *N*-aryl oxazolindine-5-carboxamide derivatives (5-9) have shown improved cytotoxicity than *N*-alkyl congeners (1-4). The oxazolindine-5-carboxamides, having *N*-phenyl (5) and *N*-(4′-fluoro)phenyl (6, IC_{50} = 5.67 μM) substituents, have showed high cytotoxicity against ovarian (PA-1) cell line while its *N*-(4′-cyano)phenyl (7) derivative exhibited high cytotoxicity against oral (KB) cell line. Compound 6 has also shown safety index > 15. The *N*-(4′-methoxy)phenyl-oxazolindine-5-carboxamide (8) elicited moderate to low cytotoxicity against a number of cancer cell lines but low

order of cytotoxicity was observed in *N*-(3′-chloro-4′-fluoro)phenyl analog (**9**). It seemed that di-substituents or electron donating group present in the phenyl ring are not good choice for enhancing the cytotoxicity. However, *N*-benzyl-oxazolindine-5-carboxamide (**10**) exhibited high cytotoxictity aganist PA-1 ($IC_{50} = 6.1\ \mu M$) and KB ($IC_{50} = 4.17\ \mu M$) cell lines with safety index > 4. The *N*-heteroaryl-oxazolindine-5-carboxamide derivatives (**11-14**) were not found superior as compared to *N*-aryl (**5-9**) or *N*-benzyl (**10**) congeners. For example, all the *N*-pyridine derivatives (**11-13**) have shown low cytotoxicity, while *N*-thiazole (**14**) analog was essentially inactive. Similarly, oxazolindine-5-carboxamides having tertiary amines as substituents, like piperidine (**15**), morpholine (**16**) and pyrrolidine (**17**), have elicited low cytotoxicity. The lower cytotoxicity was exhibited when oxazolindine ring (**1-17**) was opened to corresponding amino-alcohol derivatives (**18-34**). All the amino-alcohol derivatives (**18-34**) either showed low cytotoxicity or were found inactive, except compound **24**, which possesses a *N*-(4′-cyano)phenyl substituent, showed moderate cytotoxicity against oral cancer cell line. Anticancer activity was also tested against an colon (SW620), breast (HBL100), lung (A-549), pancreas (MIAPaCa2), leukemia (K562) and endotheial (ECV304) cancer cell lines, but none of the compounds (**1-34**) showed "high" activity.

These results clearly indicated that oxazolindines (**1-17**), in general, were found superior to their corresponding amino-alcohol derivatives (**18-34**). Amongst oxazolindine-5-carboxamide derivatives, compounds containing a *N*-aryl (**5-9**) or *N*-benzyl (**10**) substituent, exhibited high cytototoxity against ovary and oral cancers with a good safety profile. These compounds could be of use in designing new anti-cancer agents.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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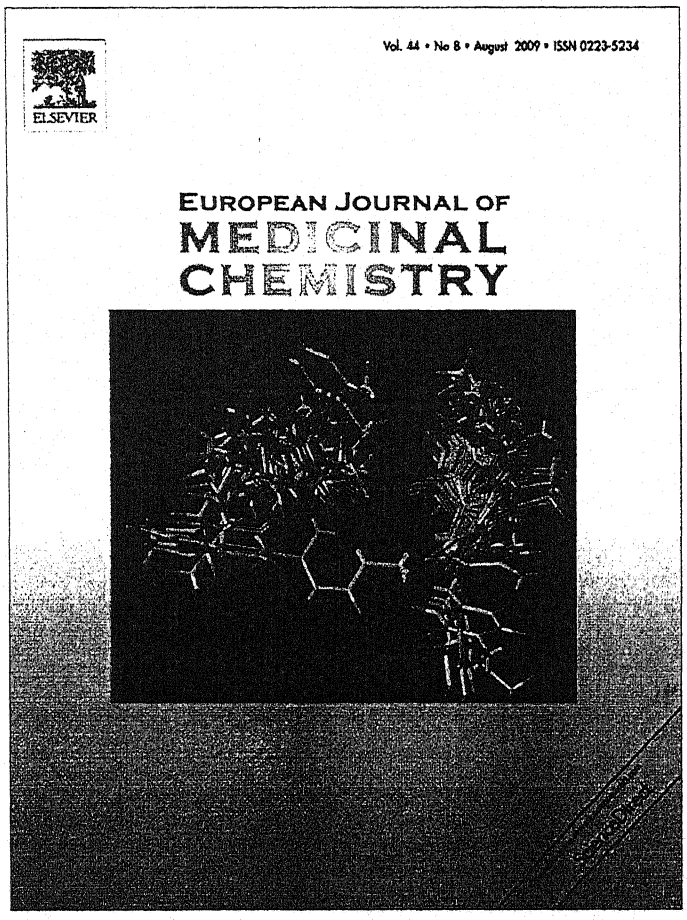

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Preliminary communication

1,8-Naphthyridine-3-carboxamide derivatives with anticancer and anti-inflammatory activity

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ABSTRACT

A number of 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**15–35**) have been synthesized and screened for their in vitro cytotoxicity and anti-inflammatory activity. Compounds **22**, **31** and **34** have shown high cytotoxicity against a number of cancer cell lines, while compound **24** showed significant anti-inflammatory activity.

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1. Introduction

Cancer, a disease of worldwide importance, according to the American Cancer Society, is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Recently, quinolines and 1,8-naphthyridine are being exploited in cancer chemotherapy and one of the molecules SNS-595 is in second phase of clinical trials [1,2]. Mammalian Topoisomerase II is one of the known targets for anti-tumor agents like doxorubicin, etoposide, ellipticine and amsacrine [3]. 1,8-Naphthyridine derivatives were found to display moderate cytotoxic activity against murine P388 leukemia, when changes were carried out at N-1 and C-7 positions [4,5]. However, further structural exploitations in 1,8-naphthyridine skeleton are required to establish a meaningful structure-activity relationship. Earlier, we have synthesized C-3 carboxamide derivatives with a spacer, which have shown good cytotoxicity along with anti-inflammatory activity [6]. Based on these observations and SAR we have further modified the C-3 carboxamide acid with different amino acid derivatives (**4a–d** and **8a–c**) to afford 1,8-naphthyridine-3-carboxamide derivatives (**15–35**). The latter being not only cytotoxic but also safer on normal cell lines vs. tumor cells. The C-3 amide linkage in 1,8-naphthyridine-3-carboxamide derivatives may provide hydrophilic interaction, while functionalized amino acids may interact with the receptors, and as a consequence, it could trigger physiological response.

Compounds (**15–35**) have shown promising anticancer activities and were further tested for their potential anti-inflammatory activity based on the molecular link between cancer and inflammation [7–9].

2. Chemistry

The synthesis of *N*-substituted 1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives **15–35** was carried out using functionalized amino acid derivatives **4a–d** and **8a–c**. The synthesis of the racemic **4a–d** and **8a–c** are described in Scheme 1. The amino group of DL-3-amino-3-phenyl propionic acid (**1**) was protected with Boc anhydride to furnish Boc substituted amino acid **2**. The coupling of **2** with appropriate amines, using EDCI–HOBt provided the respective propionamide **3a–d**. The Boc groups of **3a–d** were removed by its treatment with 50% TFA/DCM to yield the corresponding DL-*N*-substituted 3-amino-3-phenyl propionamide (**4a–d**). Similarly, DL-*N*-substituted phenyl glycinamide derivatives (**8a–d**) were prepared starting from DL-phenyl glycine (**5**).

The synthesis of compounds **15–34** is shown in Scheme 2. Commercially available 2-chloro nicotinic acid **9** was reacted with

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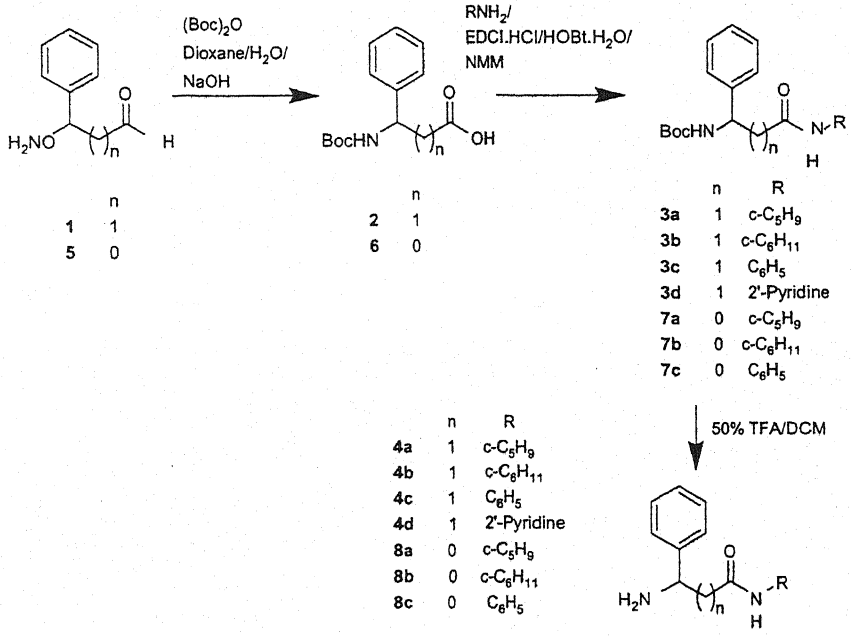
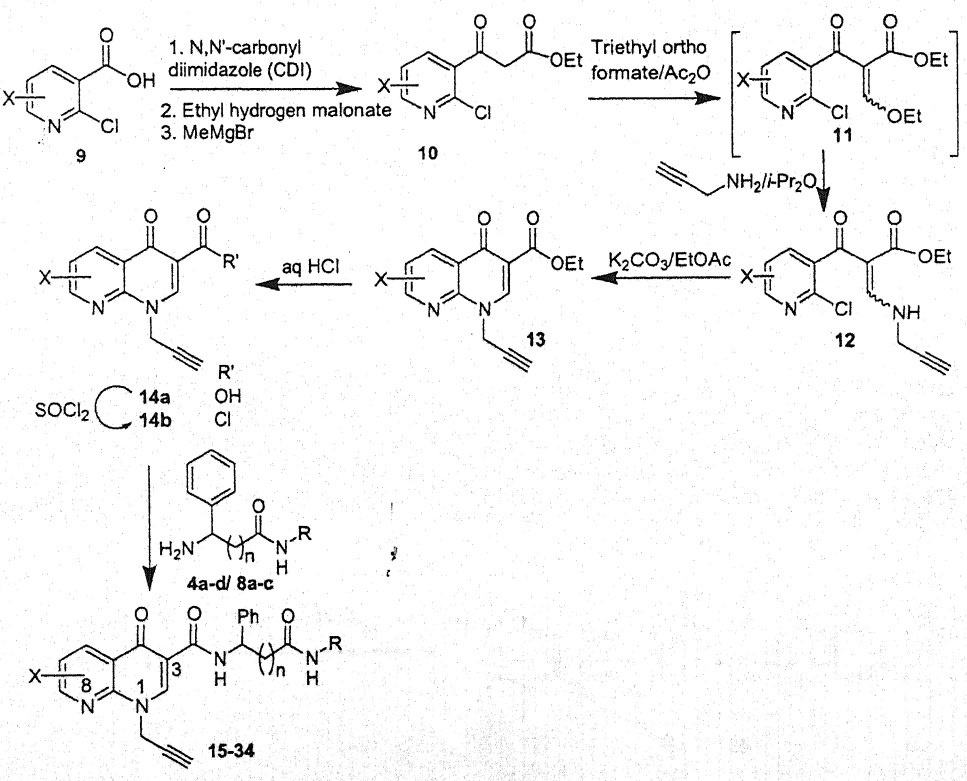


Scheme 1. Synthesis of functionalized amino acids (**4a–d** and **8a–c**).

1,1′-carbonyldiimidazole (CDI) in dry THF to afford the imidazolide solution, which was allowed to react with ethyl hydrogen malonate and methyl magnesium bromide to afford nicotinoylacetate **10**. Compound **10** on treatment with triethyl orthoformate and acetic anhydride (**11**) followed by the addition of propargyl amine afforded ethyl nicotinoylacrylate **12**. Ethyl 1,8-naphthyridine-3-carboxylate (**13**) was prepared by base-assisted (K_2CO_3) cyclization of acrylate **12** in ethyl acetate, upon acidic hydrolysis **13**; provided 1,8-naphthyridine-3-carboxylic acid **14a**, which was treated with thionyl chloride to afford **14b**. The 1-propargyl-1,8-naphthyridine-



Scheme 2. Synthesis of 1,8-naphthyridine-3-carboxamide derivatives (**15–34**).

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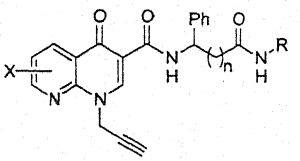
Table 1
List of 1-propargyl-1,8-naphthyridine derivatives (**15–35**).



15-35

Compound no.	X	n	R	Compound no.	X	n	R
15	H	1	c-C_5H_9	**26**	H	0	c-C_5H_9
16	H	1	c-C_6H_{11}	**27**	H	0	c-C_6H_{11}
17	H	1	C_6H_5	**28**	H	0	C_6H_5
18	H	1	2′-Pyridine	**29**	7-Cl	0	c-C_5H_9
19	7-Cl	1	c-C_5H_9	**30**	7-Cl	0	c-C_6H_{11}
20	7-Cl	1	C_6H_5	**31**	7-Cl	0	C_6H_5
21	7-Cl	1	2′-Pyridine	**32**	6-F,7-Cl	0	C_5H_9
22	6-F,7-Cl	1	c-C_5H_9	**33**	6-F,7-Cl	0	C_6H_{11}
23	6-F,7-Cl	1	c-C_6H_{11}	**34**	6-F,7-Cl	0	C_6H_5
24	6-F,7-Cl	1	C_6H_5	**35**	6-F, 7-pyrrolidine	0	C_6H_5
25	6-F,7-Cl	1	2′-Pyridine				

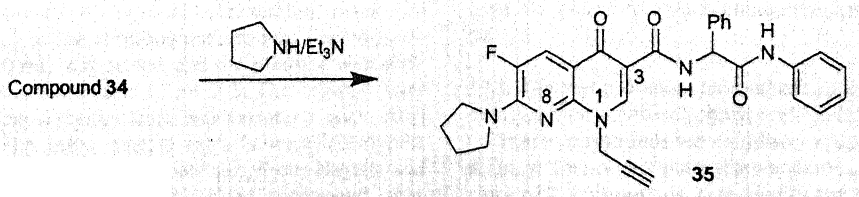
3-carboxamide derivatives **15–34** were prepared by coupling of appropriate 1,8-naphthyridine-3-carbonyl chloride (**14b**) with functionalized amino acids **4a–d** and **8a–c**, respectively. The *N*-substituted 1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives **15–34** are listed in Table 1. Compound **34** on treatment with pyrrolidine in the presence of triethylamine yielded compound **35** as shown in Scheme 3.

3. Results and discussion

The synthesized compounds (**15–35**) are divided into two classes based on the substitution of phenyl propionamide (**4a–d**) and phenyl glycinamide (**8a–c**) functionalized amino acid into *N*-(2-*N*-substituted carbomyl-1-phenylethyl)-1,4-dihydro-4-oxo -1-propargyl-1,8-naphthyridine-3-carboxamide (**15–25**) and *N*-(2-*N*-substituted carbomyl-1-phenylmethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**26–35**) derivatives, respectively. These carboxamides are further divided into three categories based on the substitution pattern at C-6 and C-7 (unsubstituted: compounds without any substitution at C-6 and C-7, monohalo substituted: C-7 chloro substituted and dihalo substituted: C-6 fluoro-C-7 chloro substituted compounds).

Amongst compounds (**15–25**), substitution in 1,8-naphthyridine ring had played crucial role in eliciting cytotoxicity. Unsubstituted 1,8-naphthyridine derivatives (**15–18**) were found inactive except compound **17**, which resulted in slight cytotoxicity on prostate cancer cell line. While, halo substituted derivatives (**19–25**) were found better than unsubstituted ones (**15–18**). The monohalo substituted cycloalkyl derivative (**19**) and its dihalo substituted analog **22** have shown high cytotoxicity on ovary cancer cell line with IC_{50} of 1.1 and 0.68 μM, respectively. Compound **22** was found as most cytotoxic molecule against breast cancer cell line with IC_{50} of 2.0 μM in this series. Upon expansion of cyclopentyl ring in compound **22** to cyclohexyl ring (**23**), cytotoxicity was lowered. The monohalo aryl substituted derivative (**20**) and its dihalo substituted analog **24** exhibited high cytotoxicity on ovarian cancer cell line. In addition, compound **20** has also shown good cytotoxicity on colon and pancreas cancer cell lines. The monohalo substituted heteroaryl derivative **21** exhibited high cytotoxicity on ovarian and colon cancer cell lines but its dihalo substituted analog **25** was found inactive against ovarian cancer cell line. However, compound **25** has resulted in high cytotoxicity against prostate, oral and colon cancer cell lines with $IC_{50} \leq 2.3$ μM. These results indicated that the halo substituted 1-propargyl-1,8-naphthyridine with 3-phenyl propionamide functionalized amino acid substitution has shown high cytotoxicity.

In second series *N*-(2-*N*-substituted carbomyl-1-phenylmethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide **26–35**, compounds **26** and **28** were found relatively better than earlier series derivatives (**15–17**). However, the monohalo substituted cycloalkyl derivatives (**29** and **30**) were found inactive. The monohalo substituted aryl derivative **31** exhibited high cytotoxicity on prostate, oral and leukemia cancer cell lines with IC_{50} of 1.7, 2.1 and 3.3 μM in this series. Similar to the earlier series dihalo derivatives (**22–24**), compounds **32–34** resulted in high cytotoxicity against a number of cancer cell lines. Compound **32** has shown broad-spectrum cytotoxicity with IC_{50} of 1.7 and 3.2 against pancreas and endothelial cancer cell lines but also exhibited cytotoxicity on normal cancer cell line. Compound **33** has shown selective and high cytotoxicity against ovarian cancer cell line. Aryl substituted phenyl derivative **34** has shown high and broad spectrum of cytotoxicity with IC_{50} of 0.5, 0.6, 1.1 and 1.4 μM against



Scheme 3. Synthesis of compound **35**.

ovarian, prostate, oral and colon cancer cell lines along with good safety index. Further, replacement of the C-7 chloro group in compound **34** with pyrrolidine (**35**) leads to complete loss of activity.

It indicated that both phenyl propionamide and phenyl glycinamide functionalized amino acid substituted 1-propargyl-1,8-naphthyridine-3-carboxamides have shown high cytotoxicity on a number of cancer cell lines particularly on ovary cancer cell line. The C-6/C-7 halo substituent in the 1,8-naphthyridine played a crucial role in eliciting cytotoxicity.

Compounds **15**, **17**, **18**, **20**, **28** & **34** exhibit >50% inhibition of IL-1-β at 1 μg/ml. >50% inhibition of IL-6 was observed by **18**, **19**, **20**, **28** & **34** at both 1 and 0.1 μg/ml. However, compounds **21**, **22**, **24**, **25** & **31** demonstrated >50% down regulation of both IL-1-β and IL-6 at 1 and 0.1 μg/ml and suggest promising anti-inflammatory activity. Compound **24** was found to be most active as it demonstrated a significant down regulation of TNF-α and IP-10 also in addition to IL-1-β & IL-6.

4. Conclusions

A number of 1,8-naphthyridine-3-carboxamide derivatives (**15–35**) have been synthesized and evaluated for their in vitro cytotoxicity and anti-inflammatory activity. Amongst them compound **34** has shown a high and broad spectrum of cytotoxicity with IC_{50} of 0.5, 0.6, 1.1 and 1.4 μM against ovarian, prostate, oral and colon cancer cell lines. Compounds **22** and **31** show significant cytotoxicity against a number of cancer cell lines, while compound **24** showed significant down regulation of TNF-α and IP-10 also in addition to IL-1-β and IL-6.

5. Experimental protocols

5.1. Chemistry

All the solvents and reagents were purchased from companies such as Aldrich, Lancaster, Acros & Rankem and were used as supplied. All TLC data (R_f values) were determined on aluminum sheets coated with silica gel 60 F_{254} (Merck). Visualization was achieved with UV light and iodine vapor. Column chromatography was performed using silica gel (100–200 mesh). ^{1}H NMR spectra were recorded on a Bruker 300 MHz instrument using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a Micromass Quattro Micro™ instrument. Elemental analyses (C, H, N) were undertaken using an elementer analyzer and were within 0.4% of the calculated values. Melting points were determined in a capillary tube with a thermal scientific melting point apparatus Mettler Toledo and are uncorrected.

5.1.1. 3-tert-*Butoxycarbonylamino-3-phenyl-propionic acid (2)*

Di-*tert*-butyl pyrocarbonate (Boc_2O, 2.4 g, 11 mmol) was added in portions to the stirred solution of (DL)-2-amino phenyl propionic acid **1** (1.65 g, 10 mmol) in dioxane (20 ml), water (10 ml) and 1 N NaOH (10 ml) at 0–5 °C. The reaction mixture was stirred at ambient temperature for 30 min and concentrated in vacuum to a volume of 10–15 ml. The resulting mixture was cooled to 0–5 °C, ethyl acetate (30 ml) was added, and the mixture was acidified with dilute hydrochloric acid to pH 2–3. The organic layer was separated and the aqueous layer was extracted with ethyl acetate (2 × 15 ml). The ethyl acetate extracts were combined, washed with water (2 × 30 ml), dried over anhydrous Na_2SO_4 and concentrated in vacuum to furnish the titled compound, m.p. 143–145 °C.

5.1.2. *General procedure for the synthesis of substituted* N-tert-*butoxycarbonylamino-3-phenyl-propionamides (3a–d)*

Appropriate amine (10 mmol) was added, to a stirred solution of (DL)-3-*tert*-butoxycarbonylamino-3-phenyl-propionic acid **2** (2.65 g, 10 mmol) in dry dichloromethane (50 ml). The resulting solution was placed in an ice bath for 15 min and 1-hydroxybenzotriazole hydrate (HOBt·H_2O, 1.84 g, 12 mmol) and *N*-methylmorpholine (NMM, 1.01 g, 10 mmol) were added. The stirring was continued for 30 min at 0 °C and 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide hydrochloride (EDCI·HCl, 1.92 g, 10 mmol) was added. The reaction mixture was stirred for 3 h at 0 °C and at rt for 5 h. Then, water (50 ml) was added to the mixture, which was extracted with dichloromethane (100 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to afford the crude residue. The crude product was used in the next step without further characterization.

5.1.3. *General procedure for the synthesis of substituted of 3-amino-3,N-phenyl-propionamide (4a–d)*

50% TFA/DCM (trifluoro acetic acid/dichloromethane, 50 ml) was added to substituted *N-tert*-butoxycarbonylamino-3-phenyl-propionamides (**3a–d**) (10 mmol) at 0 °C. The reaction mixture was stirred for 4 h at ambient temperature and then left overnight. The resulting mixture was neutralized with aqueous $NaHCO_3$ saturated solution. DCM layer was separated, dried over Na_2SO_4 and evaporated to afford the crude product. The obtained product was purified by column chromatography using 2% MeOH/DCM as eluent.

*5.1.3.1. 3-Amino-N-cyclopentyl-3-phenyl-propionamide (**4a**).* Yield: 65.4%; m.p. 113–116 °C; R_f 0.4 (10% MeOH/DCM); ^{1}H NMR (DMSO) δ 7.83 (d, 1H, J = 6.99 Hz), 7.34–7.15 (m, 5H), 4.17 (t, 1H, J = 6.80 Hz), 3.95–3.90 (m, 1H), 2.29 (d, 1H, J = 6.87 Hz), 1.72–1.22 (m, 8H); MS (ES+) 233 (M + H).

*5.1.3.2. 3-Amino-N-cyclohexyl-3-phenyl-propionamide (**4b**).* Yield: 72.4%; m.p. 137–139 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 7.40–7.26 (m, 5H), 6.67 (d, 1H, J = 7,1 Hz), 4.53 (s, 1H), 3.79–3.76 (m, 1H), 2.75–2.73 (m, 2H), 2.32 (bs, 2H), 1.84–1.59 (m, 5H), 1.42–1.05 (m, 5H); MS (ES+) 247 (M + H).

*5.1.3.3. 3-Amino-3,N-diphenyl-propionamide (**4c**).* Yield: 61.3%; m.p. 113–116 °C; R_f 0.5 (10% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.05 (s, 1H), 7.54 (d, 2H, J = 7.64 Hz), 7.38 (d, 2H, J = 7.26 Hz), 7.30–7.16 (m, 5H), 7.02–6.97 (m, 1H), 4.30–4.26 (m, 1H), 2.56–2.45 (m, 2H); MS (ES+) 241 (M + H).

*5.1.3.4. 3-Amino-3-phenyl-N-pyridin-2-yl-propionamide (**4d**).* Yield: 50.4%; m.p. 108–110 °C; R_f 0.2 (10% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 8.24 (d, 1H, J = 8.25 Hz), 7.85 (d, 1H, J = 4.44 Hz), 7.70 (t, 1H, J = 8.0 Hz), 7.45 (m, 2H), 7.44–7.31 (m, 3H), 4.30 (s, 1H), 2.73–2.71 (m, 2H), 1.66 (bs, 1H); MS (ES+) 242 (M + H).

5.1.4. N-tert-*Butoxycarbonylamino-2-phenylglycine (6)*

It was prepared in a similar manner as described for the synthesis of **2** except (DL)-2-phenylglycine (1.51 g, 10 mmol) was used in place of (DL)-3-aminophenylpropionic acid, m.p. 90–92 °C.

5.1.5. *General procedure for synthesis of 2-*N-tert-*butoxycarbonylamino-2-phenyl acetamides (7a–c)*

These compounds were similarly prepared as described for **3a–d**, from (DL)-*N-tert*-butoxycarbonylamino-2-phenylglycine (**6**) in place of **2**. Compounds were used in the next step without further characterization.

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*5.1.6. General procedure for the synthesis of 2-amino-2-phenyl acetamides (**8a–c**)*

These compounds were similarly prepared as described for **4a–d**, from 2-*N*-*tert*-butoxycarbonylamino-2-phenyl acetamides (**7a–c**) in place of **3a–d**.

*5.1.6.1. 2-Amino-N-cyclopentyl-2-phenyl acetamide (**8a**).* Yield: 68.9%; m.p. 178–180 °C; R_f 0.6 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 8.62 (s, 1H), 7.42–7.26 (m, 5H), 5.55 (d, 1H, J = 7.96 Hz), 5.11 (s, 1H), 3.78–3.69 (m, 1H), 1.76–1.00 (m, 8H); MS (ES+) 219 (M + H).

*5.1.6.2. 2-Amino-N-cyclohexyl-2-phenyl acetamide (**8b**).* Yield: 65.3%; m.p. 91–93 °C; R_f 0.4 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 7.86 (d, 1H, J = 7.86 Hz), 7.36–7.17 (m, 5H), 4.27 (s, 1H), 2.18 (s, 1H), 1.72–1.10 (m, 10H); MS (ES+) 233 (M + H).

*5.1.6.3. 2-Amino-2,N-diphenyl acetamide (**8c**).* Yield: 53.2%; m.p. 112–114 °C; R_f 0.4 (7% MeOH/DCM); ^{1}H NMR (DMSO) δ 9.99 (bs, 1H), 7.62–7.21 (m, 9H), 7.02 (t, 1H, J = 7.3 Hz), 4.51 (s, 1H); MS (ES+) 225 (M + H).

*5.1.7. General procedure for the synthesis of N-substituted 1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**15–34**)*

Thionyl chloride (15 mmol) was added dropwise to a stirred solution of 1-propargyl-1,8-naphthyridine-3-carboxylic acid (**14a**, 10 mmol) in dichloromethane (50 ml). The stirring was continued for 4 h at room temperature and dried under vacuum to provide acid chloride intermediate **14b**. Compound **14b** was diluted with dichloromethane (50 ml) and appropriate amine (15 mmol) was added to it and stirred for 2 h. To the reaction mixture was added water (50 ml), which was extracted with dichloromethane (100 ml). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to dryness to provide crude product. The obtained crude product was purified over silica column using MeOH/DCM as eluent, to furnish the desired pure compound.

*5.1.7.1. N-(2-N-Cyclopentylcarbomyl-1-phenylethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**15**).* Yield: 39%; m.p. 193–195 °C; R_f 0.5 (7% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.61 (d, 1H, J = 8.1 Hz), 9.18 (s, 1H), 8.82–8.79 (2H, m), 7.50–7.24 (m, 6H), 5.88 (d, 1H, J = 7.1 Hz), 5.61 (dd, 1H, J = 6.63 Hz), 5.28 (s, 2H), 4.18 (m, 1H), 2.81–2.78 (m, 2H), 2.53 (m, 1H), 1.87–1.49 (m, 6H), 1.28–1.23 (m, 2H); MS (ES+) 443 (M + H).

*5.1.7.2. N-(2-N-Cyclohexylcarbomyl-1-phenylethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**16**).* Yield: 62.7%; m.p. 208–210 °C; R_f 0.3 (7% Acetone/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.64 (d, 1H, J = 8.1 Hz), 9.17 (s, 1H), 8.83–8.81 (m, 2H), 7.50–7.22 (m, 6H), 5.70 (m, 2H), 5.28 (s, 2H), 3.75–3.64 (m, 1H), 2.80 (m, 2H), 2.52 (m, 1H), 1.77–0.87 (m, 10H); MS (ES+) 457 (M + H).

*5.1.7.3. N-(2-N-Phenylcarbomyl-1-phenylethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**17**).* Yield: 44.1%; m.p. 185–187 °C; R_f 0.4 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.68 (d, 1H, J = 7.65 Hz), 9.20 (s, 1H), 8.82–8.78 (m, 2H), 8.24 (s, 1H), 7.60–7.18 (m, 10H), 7.07–7.03 (m, 1H), 5.72 (q, 1H, J = 7.9, 13.6 Hz), 5.35–5.22 (m, 2H), 3.14–2.97 (m, 2H), 2.53 (s, 1H); MS (ES+) 451 (M + H).

*5.1.7.4. N-(2-N-Pyridin-2′-ylcarbomyl-1-phenylethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**18**).* Yield: 38.4%; m.p. 228–230 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.56 (s, 1H), 10.35 (d, 1H, J = 8.37), 9.12 (1H, s), 8.96–8.94 (m, 1H), 8.70 (dd, 1H, J = 1.86 Hz), 8.26 (d, 1H, J = 3.84 Hz), 8.02 (d, 1H, J = 8.4 Hz), 7.74–7.64 (m, 2H), 7.43–7.32 (m, 4H), 7.26–7.21 (m, 1H), 7.05–7.03 (m, 1H), 5.60 (q, 1H, J = 7.59, 14.8), 5.40 (d, 2H, J = 2.37 Hz), 3.51–3.49 (m, 1H), 3.09–2.97 (m, 2H); MS (ES+) 452 (M + H).

*5.1.7.5. N-(2-N-Cyclopentylcarbomyl-1-phenylethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**19**).* Yield: 55.4%; m.p. 208–210 °C; R_f 0.6 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.57 (d, 1H, J = 8.01 Hz), 9.15 (s, 1H), 8.73 (d, 1H, J = 8.3), 7.46–7.22 (m, 5H), 5.75 (d, 1H, J = 7.37), 5.60 (q, 1H, J = 6.7, 14.5 Hz), 5.22–5.21 (m, 2H), 4.17 (q, 1H, J = 6.7, 13.5), 2.78 (d, 2H, J = 6.61), 2.56–2.54 (m, 1H), 1.87–1.79 (m, 2H), 1.59–1.51 (m, 4H), 1.26–1.20 (m, 2H); MS (ES+) 477 (M + H).

*5.1.7.6. N-(2-N-Phenylcarbomyl-1-phenylethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**20**).* Yield: 50.4%; m.p. 220–222 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.59 (d, 1H, J = 8.0 Hz), 9.17 (s, 1H), 8.70 (d, 1H, J = 8.3), 8.16 (s, 1H), 7.51–7.02 (m, 11H), 5.71 (d, 1H, J = 6.1), 5.21 (s, 2H) 3.07–3.00 (m, 2H), 2.56 (s, 1H); MS (ES+) 485 (M + H).

*5.1.7.7. N-(2-N-Pyridin-2′-ylcarbomyl-1-phenylethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**21**).* Yield: 52.7%; m.p. 208–210 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.58 (s, 1H), 10.27 (d, 1H, J = 8.34 Hz), 9.10 (s, 1H), 9.08 (s, 1H), 8.67 (d, 1H, J = 8.31 Hz), 8.27 (d, 1H, J = 3.6), 8.01 (d, 1H, J = 8.34 Hz), 7.74–7.69 (m, 2H), 7.42–7.24 (m, 5H), 7.05 (m, 1H), 5.60 (q, 1H, J = 7.3, 14.7), 5.30–5.31 (m, 1H), 3.57 (t, 1H, J = 2.3 Hz), 3.12–2.96 (m, 2H); MS (ES+) 486 (M + H).

*5.1.7.8. N-(2-N-Cyclopentylcarbomyl-1-phenylethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**22**).* Yield: 67.6%; m.p. 208–210 °C; R_f 0.6 (5% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.52 (d, 1H, J = 8.07 Hz), 9.15 (s, 1H), 8.50 (d, 1H, J = 7.32 Hz), 7.42–7.22 (m, 5H), 5.69 (d, 1H, J = 7.26 Hz), 5.58 (q, 1H, J = 6.7 Hz, 14.31 Hz), 5.20 (s, 2H), 4.16 (q, 1H, J = 6.9 Hz, 13.6 Hz), 2.78–2.76 (m, 2H), 2.56 (d, 1H, J = 2.5 Hz), 1.87–1.19 (m, 8H); MS (ES+) 495 (M + H).

*5.1.7.9. N-(2-N-Cyclohexylcarbomyl-1-phenylethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**23**).* Yield: 67.5%; m.p. 179–181 °C; R_f 0.3 (7% Acetone/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.57 (d, 1H, J = 8.1 Hz), 9.14 (s, 1H), 8.51 (d, 1H, J = 7.32 Hz), 7.42–7.22 (m, 5H), 5.60–5.51 (m, 2H), 5.20 (d, 2H, J = 2.5 Hz), 3.75–3.72 (m, 1H), 2.77–2.75 (m, 2H), 2.5 (q, 1H, J = 2.5 Hz), 1.73–1.5 (m, 4H), 1.30–0.94 (m, 6H); MS (ES+) 509 (M + H).

*5.1.7.10. N-(2-N-Phenylcarbomyl-1-phenylethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**24**).* Yield: 50.7%; m.p. 171–173 °C; R_f 0.6 (7% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.28 (d, 1H, J = 8.2 Hz), 9.98 (s, 1H), 9.1 (1H, s), 8.62 (d, 1H, J = 7.8 Hz), 7.50 (d, 2H, J = 7.8 Hz), 7.40–7.23 (m, 5H), 7.13–7.11 (m, 3H), 6.98 (t, 1H, J = 7.2 Hz), 5.59 (q, 1H, J = 7.1, 14.2 Hz), 5.30 (s, 1H), 3.56 (s, 1H), 3.01–2.85 (m, 2H); MS (ES+) 503 (M + H).

*5.1.7.11. N-(2-N-Pyridin-2′-ylcarbomyl-1-phenylethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide (**25**).* Yield: 37.0%; m.p. 196–198 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.56 (s, 1H), 10.23 (d, 1H, J = 8.34 Hz), 9.10 (s, 1H), 8.60 (d, 1H, J = 7.8 Hz), 8.25 (d, 1H, J = 4.14 Hz), 8.0 (d, 1H, J = 8.34 Hz), 7.69 (m, 1H), 7.40–7.30 (m, 4H), 7.22 (t, 1H, J = 7.14 Hz), 7.04 (q, 1H, J = 5.2, 6.8 Hz), 5.58 (q, 1H, J = 7.4, 14.2 Hz), 5.31–5.30 (d, 2H, J = 2.1 Hz), 3.56 (t, 1H, J = 2.14 Hz), 3.10–2.95 (m, 2H); MS (ES+) 504 (M + H).

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5.1.7.12. N-*(2-N-Cyclopentylcarbomyl-1-phenylmethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (**26**). Yield: 52.5%; m.p. 215–217 °C; R_f 0.4 (2% MeOH/DCM); ^{1}H NMR ($CDCl_3$) δ 10.77 (d, 1H, J = 6.5 Hz), 9.16 (s, 1H), 8.81 (d, 1H, J = 6.6 Hz), 7.51–7.30 (m, 5H), 5.83 (d, 1H, J = 6.7 Hz), 5.61 (d, 1H, J = 6.9 Hz), 5.27 (q, 2H, J = 2.4, 4.8 Hz), 4.22 (d, 2H, J = 6.9 Hz), 2.52 (d, 2H, J = 2.3 Hz), 1.97–1.92 (m, 2H), 1.60–1.25 (m, 6H); MS (ES+) 429 (M + H).

5.1.7.13. N-*(2-N-Cyclohexylcarbomyl-1-phenylmethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (**27**). Yield: 65.6%; m.p. 206–208 °C; R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.66 (d, 1H, J = 6.21 Hz), 9.16 (s, 1H), 8.81 (d, 2H, J = 6.7 Hz), 7.51–7.26 (m, 6H), 5.70 (d, 2H, J = 8.3 Hz), 5.61 (d, 1H, J = 7.09 Hz), 5.28–5.20 (m, 2H), 3.81–3.75 (m, 1H), 2.80–2.78 (m, 1H), 2.51 (d, 1H, J = 2.5 Hz), 2.02–1.63 (m, 3H) 1.63–1.02 (m, 7H); MS (ES+) 443 (M + H).

5.1.7.14. N-*(2-N-Phenylcarbomyl-1-phenylmethyl)-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (**28**). Yield: 66.0%; m.p. 187–189 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.73 (d, 1H, J = 5.82 Hz), 10.51 (s, 1H), 9.15 (s, 1H), 8.96 (s, 1H), 8.73 (d, 1H, J = 7.35 Hz), 7.68–7.29 (m, 10H), 7.04 (s, 1H), 5.89 (d, 1H, J = 7.4 Hz), 5.41 (bs, 2H), 3.51 (s, 1H); MS (ES+) 437 (M + H).

5.1.7.15. N-*(2-N-Cyclopentylcarbomyl-1-phenylmethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (**29**). Yield: 75.5%; m.p. 191–193 °C; R_f 0.3 (2% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.52 (s, 1H), 9.09 (s, 1H), 8.69 (d, 1H, J = 7.02 Hz), 8.38 (d, 1H, J = 9.36 Hz), 7.70 (d, 1H, J = 9.75 Hz), 7.40–7.27 (m, 5H), 5.65 (s, 1H), 5.34–5.27 (m, 2H), 3.93 (s, 1H), 3.54–3.52 (m, 1H), 1.82–1.11 (m, 8H); MS (ES+) 463 (M + H).

5.1.7.16. N-*(2-N-Cyclohexylcarbomyl-1-phenylmethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (**30**). Yield: 64.4%; m.p. >250 °C; R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}H NMR (DMSO) δ 10.52 (d, 1H, J = 7.8 Hz), 9.09 (s, 1H), 8.69 (d, 1H, J = 8.4 Hz), 8.32 (d, 1H, J = 7.74 Hz), 7.71 (d, 1H, J = 8.2 Hz), 7.43–7.16 (m, 5H), 5.68 (d, 1H, J = 7.8 Hz), 5.30 (d, 2H, J = 1.83 Hz), 3.55 (s, 2H), 1.78–1.49 (m, 5H), 1.26–0.83 (m, 5H); MS (ES+) 477 (M + H).

5.1.7.17. N-*(2-N-Phenylcarbomyl-1-phenylmethyl)-7-chloro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (**31**). Yield: 65.1%; m.p. >250 °C; R_f 0.5 (5% MeOH/DCM); ^{1}H NMR (DMSO) δ 10.66 (d, 1H, J = 7.5 Hz), 10.50 (s, 1H), 9.14 (s, 1H), 8.72 (d, 1H, J = 8.37 Hz), 7.73 (d, 1H, J = 8.34 Hz), 7.61–7.54 (m, 4H), 7.43–7.28 (m, 5H), 7.08–7.03 (m, 1H), 5.89 (d, 2H, J = 7.4 Hz), 5.33 (d, 1H, J = 1.58 Hz), 3.58 (s, 1H); MS (ES+) 471 (M + H).

5.1.7.18. N-*(2-N-Cyclopentylcarbomyl-1-phenylmethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (**32**). Yield: 65.0%; m.p. 238–240 °C; R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}H NMR (DMSO) δ 10.41 (d, 1H, J = 7.83 Hz), 9.04 (s, 1H), 8.57 (d, 1H, J = 7.83 Hz), 8.32 (d, 1H, J = 7.17 Hz), 7.34 (d, 2H, J = 7.35 Hz), 7.28–7.16 (m, 3H), 5.58 (d, 1H, J = 7.8 Hz), 5.25 (d, 2H, J = 2.3 Hz), 3.93–3.84 (m, 1H), 3.50 (t, 1H, J = 2.3 Hz), 1.63–1.17 (m, 8H); MS (ES+) 481 (M + H).

5.1.7.19. N-*(2-N-Cyclohexylcarbomyl-1-phenylmethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (**33**). Yield: 57.1%; m.p. >250 °C; R_f 0.5 (7% Acetone/$CHCl_3$); ^{1}H NMR (DMSO) δ 10.47 (d, 1H, J = 8.31 Hz), 9.11 (s, 1H), 8.64 (d, 1H, J = 6.18 Hz), 8.31 (d, 1H, J = 7.89 Hz), 7.43–7.25 (m, 5H), 5.67 (d, 1H, J = 7.65 Hz), 5.32 (d, 2H, J = 2.3 Hz), 3.56–3.48 (m, 2H), 1.77–1.04 (m, 10H); MS (ES+) 495 (M + H).

5.1.7.20. N-*(2-N-Phenylcarbomyl-1-phenylmethyl)-7-chloro-6-fluoro-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (**34**). Yield: 52.8%; m.p. >250 °C; R_f 0.4 (7% Acetone/$CHCl_3$); ^{1}H NMR (DMSO) δ 10.61 (d, 1H, J = 7.5 Hz), 10.49 (s, 1H), 9.1 (s, 1H), 8.66 (d, 1H, J = 7.7 Hz), 7.59–7.45 (m, 4H), 7.41–7.26 (m, 5H), 7.07–7.02 (m, 1H), 5.87 (d, 1H, J = 7.5 Hz), 5.32 (d, 2H, J = 2.3 Hz), 3.57 (d, 1H, J = 2.38 Hz); MS (ES+) 489 (M + H).

5.1.7.21. N-*(2-N-Phenylcarbomyl-1-phenylmethyl)-6-fluoro-7-pyrrolidinyl-1,4-dihydro-4-oxo-1-propargyl-1,8-naphthyridine-3-carboxamide* (**35**). Triethylamine (1.1 g, 11 mmol) and pyrrolidine (2.13 g, 30 mmol) were added to a suspension of compound **34** (4.89 g, 10 mmol) in acetonitrile (50 ml) and refluxed for 3 h. The reaction mixture was cooled; the precipitate thus separated was collected by filtration, washed with acetonitrile and dried to give compound **35**. Yield 4.52 g (86.3 %). m.p. >250 °C; R_f 0.4 (2% Methanol/DCM); ^{1}H NMR (DMSO) δ 10.98 (d, 1H, J = 7.4 Hz), 10.45 (s, 1H), 8.8 (s, 1H), 7.98–7.94 (m, 1H), 7.59–7.26 (m, 9H), 7.04 (s, 1H), 5.85 (d, 1H, J = 7.9 Hz), 5.23 (s, 2H), 3.75 (bs, 4H), 3.45 (s, 1H), 1.94 (s, 4H); MS (ES+) 523 (M + H).

5.2. Biological activity

5.2.1. Cytotoxicity

All the synthesized derivatives **15–35** were tested for in vitro cytotoxicity on nine cancerous as well as a non-cancerous cell lines and IC_{50} values were calculated in micromole (μM) [10]. The human cancer cell lines used in the study are ovary (PA1), prostate (DU145), oral (KB), colon (SW620), breast (HBL100), lung (A549), pancreas (MIAPaCa2), leukemia (K562) and endothelial (ECV304) cancer. All the 1,8-naphthyridine **15–35** and assay standard Doxorubicin HCl were also tested against normal mouse fibroblast (NIH3T3) cell line to evaluate their cancer cell specificity (safety index). The cytotoxicity data are summarized in Table 2. Compounds, which were found inactive, are not listed in Table 2. Derivatives of 1,8-naphthyridine-3-carboxamide (**15–35**) were screened for cytotoxic activity at the highest soluble concentration of 10 μM and on four lower concentrations on nine human tumor and one non-tumorous cell lines.

Briefly, a three days MTT in vitro cytotoxicity assay was performed, which is based on the principle of uptake of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), a tetrazolium salt by the metabolically active cells. MTT is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically at 540 nm. MTT solution (5 mg/ml) was prepared in phosphate buffered saline, pH 7.4 and filtered through a 0.22-μm filter. For each type of tumor and normal cell, 5000–10,000 cells were seeded in a 96-well culture plate and treated with various concentrations of 1,8-naphthyridine-3-carboxamide derivatives (**15–35**) for an incubation period of 72 h in CO_2 incubator. Control cells were not treated with 1,8-naphthyridine-3-carboxamide derivatives. The assay was terminated after 72 h by adding 125 μg (25 μl) MTT to each well. After incubation for 3 h, 50 μl of 10% SDS–0.01 N HCl was added to each well to lyse the cells and dissolve formazan. Plate was read spectrophotometrically at 540 nm after 1 h. Cytotoxicity percentage was calculated using the following formula: Cytotoxicity percentage = $(1 - (X/R_1)) \times 100$, where X = (absorbance of treated sample at 540 nm) − (absorbance of blank at 540 nm), R_1 = absorbance of control sample at 540 nm.

5.2.2. Anti-inflammatory activity

Dendritic cells (DCs) are central to an immune response and function as the best antigen-presenting cells. DCs have been identified as the cellular target for understanding pharmacological role of various immunomodulatory agents [11,12]. Pro-inflammatory

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Table 2
In vitro cytotoxicity of 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (**15–35**).

Compound no.	IC_{50} (μM)									
	PA1 (ovary)	DU145 (prostate)	KB (oral)	SW620 (colon)	HBL100 (breast)	A549 (lung)	MIAPaCa2 (pancreas)	K562 (leukemia)	ECV304 (endothelial)	NIH3T3 (normal fibroblast)
Doxorubicin	0.63	0.10	3.0	0.08	0.24	0.08	0.15	0.10	NA	0.39
17	>10	7.2	>10	>10	>10	>10	>10	>10	>10	NA
19	1.1	>10	>10	2.7	3.2	9.5	2.4	6.8	7.8	4.4
20	0.54	>10	>10	2.9	4.0	>10	3.0	7.7	4.0	4.4
21	1.7	4.9	5.2	2.9	>10	>10	>10	5.9	>10	NA
22	0.68	>10	4.9	2.1	2.0	6.1	4.4	7.3	5.1	2.4
23	2.3	8.03	>10	>10	>10	>10	4.9	6.2	9.8	NA
24	2.1	5.9	>10	5.7	9.3	>10	6.9	6.6	>10	9.7
25	>10	2.1	2.3	2.3	>10	>10	>10	>10	>10	4.2
26	>10	>10	4.5	>10	>10	>10	>10	>10	>10	NA
28	8.9	8.8	3.0	>10	>10	>10	>10	>10	>10	NA
31	>10	1.7	2.1	2.2	7.8	>10	9.7	3.3	>10	NA
32	1.8	3.2	3.5	3.4	5.1	>10	1.7	5.9	3.2	0.4
33	1.8	>10	>10	>10	>10	>10	>10	>10	>10	7.4
34	0.5	0.6	1.1	1.4	>10	>10	>10	5.0	9.1	NA

NA – not active. Cytotoxicity was assessed by MTT assay as described in Methods. Data shown are IC_{50} of single independent experiments done in triplicate. If IC_{50} was not achieved even at the highest concentration tested i.e. 10 μM, it was represented as NA.

Table 3
Down regulation of IL-1-β and IL-6 activity by 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (% change calculated with reference to LPS stimulated levels secreted by DCs).

Compound no.	IL-1-β (% inhibition)		IL-6 (% inhibition)	
	1 μg/ml	0.1 μg/ml	1 μg/ml	0.1 μg/ml
15	−52.1	−62.4	–	–
17	−78.5	−75.1	–	–
18	−50.1	−34.1	−74.9	−74.4
19	−18.1	−82.0	−81.7	−79.8
20	−99.4	−28.3	−75.4	−69.5
21	−82.0	−57.3	−82.9	−78.2
22	−102.0	−83.4	−100.0	−80.1
24	−106.8	−84.8	−96.0	−60.0
25	−109.6	−83.4	−99.1	−75.5
28	−64.6	−39.9	−79.3	−79.3
31	−86.3	−82.0	−91.7	−76.5
34	−97.0	−44.3	−99.9	−84.4

Table 4
Down regulation of TNF-α and IP-10 activity by 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives (% change calculated with reference to LPS stimulated levels secreted by DCs).

Compound no.	TNF-α (% inhibition)		IP-10 (% inhibition)	
	1 μg/ml	0.1 μg/ml	1 μg/ml	0.1 μg/ml
15	−34.7	−19.3	−25.0	−38.8
17	−29.7	−19.1	−33.1	−31.7
24	−99.0	−80.0	−102.4	−17.3

Cytokine levels were estimated by ELISA as described in Methods. Data shown are percent inhibition of cytokine/chemokine in duplicate.

cytokines are being explored as potential targets in therapeutic interventions for various inflammatory disorders such as Rheumatoid Arthritis [13]. In the present study, various derivatives were evaluated for down regulation of IL-1-β, IL-6, TNF-α and IP-10 levels secreted by LPS stimulated DCs.

Primary DC cultures were generated from femoral bone marrow of 8–12 weeks old C57BL/6 mice [14]. Percent change in cytokine/chemokine = $\{(B - A)/A \times 100$, where B = concentration of cytokine/chemokine (pg/ml) secreted by LPS stimulated DCs when incubated with test molecule, A = concentration of cytokine/chemokine (pg/ml) secreted by LPS stimulated DCs alone. Bone marrow progenitors were cultured in RPMI-1640 supplemented with 10% FBS (Hyclone) and 20 ng/ml rmGMCSF (R&D Systems, MN, USA) at 37 °C, 5% CO_2. Immature DCs at day 6 were stimulated with 10 ng/ml lipopolysaccharide (LPS; SIGMA) and incubated with the 1-propargyl-1,8-naphthyridine-3-carboxamide derivatives at two concentrations, 0.1 and 1 μg/ml for 24 h. The IL-1-β and IL-6, TNF-α and IP-10 secreted by the DCs were measured in culture supernatants by Enzyme Linked Immunosorbent Assays (R&D Systems Inc, MN, USA). Compounds showing down regulation of one or more of the cytokines or chemokines by >25% were considered to have potential anti-inflammatory activity as shown in Tables 3 and 4.

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RESEARCH ARTICLE

Anticancer and immunomodulatory activities of novel 1,8-naphthyridine derivatives

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Abstract

A number of 1,8-naphthyridine derivatives (**22–62**) have been synthesized and screened for their *in vitro* cytotoxicity against eight tumors and two non-tumor cell lines. Halogen substituted 1,8-naphthyridine-3-caboxamide derivatives showed potent activity with compound **47** having IC_{50} of 0.41 and 0.77 μM on MIAPaCa and K-562 cancer cell lines, respectively while, compound **36** had IC_{50} of 1.19 μM on PA-1 cancer cell line. However, one of the unsubstituted 1,8-naphthyridine-C-3'-heteroaryl derivative **29** showed potent cytotoxicity with IC_{50} of 0.41 and 1.4 μM on PA-1 and SW620 cancer cell lines, respectively. These compounds were also evaluated for anti-inflammatory activity as suggested by downregulation of proinflammaotory cytokines.

Keywords: *Anticancer; anti-inflammatory; naphthyridine*

Introduction

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. Recently 1,8-naphthyridine is being exploited in cancer chemotherapy like SNS-595 (Figure 1), which is in second phase of clinical trial [1–4]. In our efforts to find out a potent molecule, we have modified the C-3 carboxylic acid of 1,8-naphthyridine with different non-conventional functionalized amino acids, which were synthesized "in house" to afford 1,8-naphthyridine-3-carboxamide derivatives (22–62) [5].

Mammalian Topoisomerase II is one of the known target for antitumor agents like doxorubicin, etoposide, ellipticine and amascrine [6]. 1,8-Naphtyridine derivatives were found to display moderate cytotoxic activity against murine P388 leukemia, when changes were carried out at N-1 and C-7 position [3,4]. We have carried out further changes at C-3 position and synthesized 1,8-naphthyridine-3-carboxamide derivatives (22–62), and tested them against different cancer cell lines. These compounds have shown promising anticancer activities and were further tested for their potential anti-inflammatory activity based on the molecular link between cancer and inflammation [7–9]. An *in vitro* septic shock assay based on murine bone marrow-DCs has been used to evaluate potential anti-inflammatory activity as indicated by resultant down regulation of various pro-inflammatory cytokines.

Materials and methods

All the solvents and reagents were purchased from Aldrich, Lancaster or Across & Rankem and were used as supplied. All TLC data (R_f values) were determined on aluminum sheets coated with silica gel 60 F_{254} (Merck) and visualization was achieved with UV light and iodine vapors. Column chromatography was performed using silica gel (100–200 mesh) and the synthesized compounds were characterized using ^1H NMR and mass spectroscopy. Proton Magnetic Resonance (^1H NMR) spectra were recorded on a Bruker 300 MHz instrument using tetramethylsilane (TMS) as an internal standard and mass spectra were recorded on a Micromass Quattro Micro™ instrument. The purity of the synthesized compounds was determined on a Shimadzu HPLC LC-2010 C HT instrument using a gradient system. Melting points were obtained

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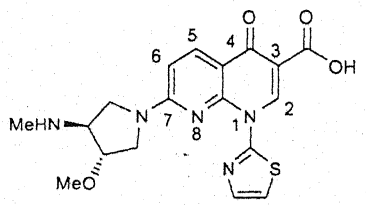
in a capillary tube with a thermal scientific melting point apparatus Mettler Toledo and are uncorrected.

Chemistry

Commercially available, 2-chloro nicotinic acid **1** was reacted with 1,1'-carbonyldiimidazole (CDI), ethyl hydrogen malonate and methyl magnesium bromide in dry THF to afford nicotinoylacetate **2**. Compounds **2** upon treatment with triethyl orthoformate and acetic anhydride followed by reaction with propargyl amine, afforded ethyl nicotinoylacrylate **19**. Further cyclization of the compound **19** using K_2CO_3 in ethyl acetate provided ethyl 1,8-naphthyridine-3-carboxylate (**20**), Compound **20** on acidic hydrolysis resulted in 1,8-naphthyridine-3-carboxylic acids (**21**). The acid **21** was treated with different functionalized amino acids **3–18** (Table 1), prepared in-house, to afford 1,8-naphthyridine-3-carboxamide derivatives **22–62** (Table 2) as shown in Schemes 1 and 2.

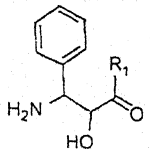
4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopropylcarbamoyl-2-hydroxy-1-



SNS-595

Figure 1. Structure of reference compound.

Table 1. Functionalised amino acid derivatives (**3–18**).



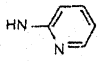
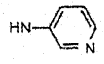
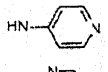
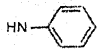
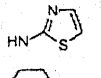
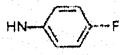
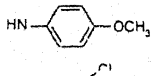
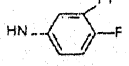
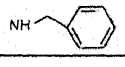
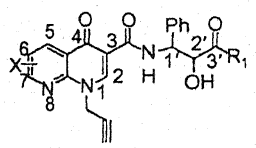
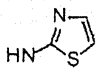
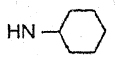
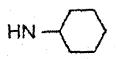
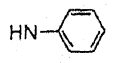
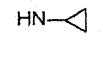
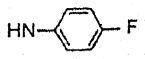
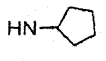
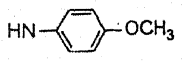
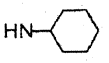
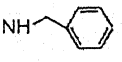
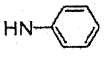
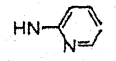
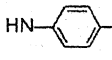
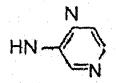
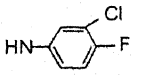
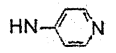
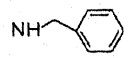
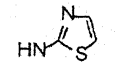
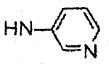
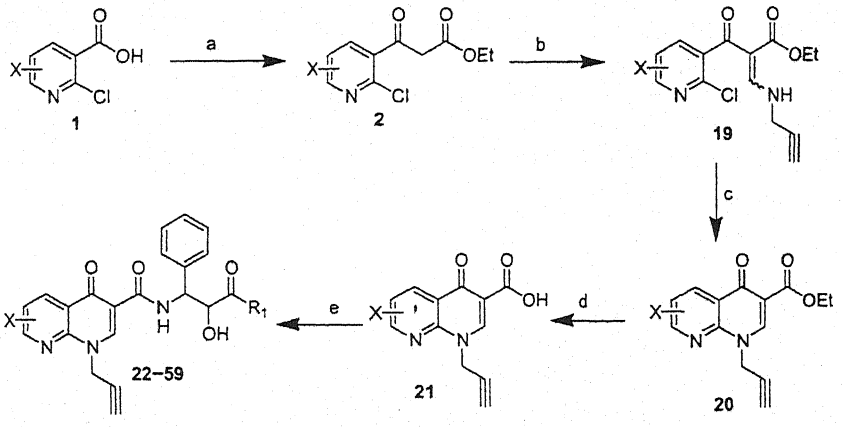
Compound No.	R_1	Compound No.	R_1
3		11	
4		12	
5		13	
6		14	
7		15	
8		16	
9		17	
10		18	OC_2H_5

Table 2. 1,8-Naphthyridine derivatives (**22–62**).

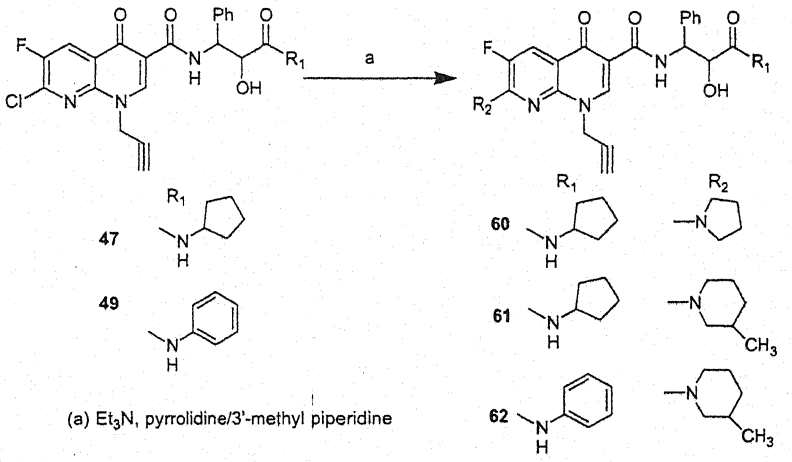


C. No.	R_1	X	C. No.	R_1	X
22		H	43		7-Cl
23		H	44		7-Cl
24		H	45		7-Cl
25		H	46		7-Cl
26		H	47		6-F, 7-Cl
27		H	48		6-F, 7-Cl
28		H	49		6-F, 7-Cl
29		H	50		6-F, 7-Cl
30		H	51		6-F, 7-Cl
31		H	52		6-F, 7-Cl
32		H	53		6-F, 7-Cl
33		H	54		6-F, 7-Cl
34		H	55		6-F, 7-Cl
35		7-Cl	56		6-F, 7-Cl
36		7-Cl	57		6-F, 7-Cl
37		7-Cl	58		7-CH_3
38		7-Cl	59	OCH_2CH_3	H
39		7-Cl	60		6-F, 7-pyrrolidine
40		7-Cl	61		6-F, 7-(3"-methylpiperidine)
41		7-Cl	62		6-F, 7-(3"-methylpiperidine)
42		7-Cl			



X = H/6-Cl/5-F, 6-Cl/6-CH_3
(a) (1) CDI; (2) $EtOCOCH_2COOH$, MeMgBr; (b) (1) $(EtO)_3CH$, Ac_2O (2) propargylamine; (c) K_2CO_3; (d) aq.HCl
(e) (1)$SOCl_2$ (2) **3–18**

Scheme 1. Synthesis of tested compounds (**22–59**).



(a) Et_3N, pyrrolidine/3'-methyl piperidine

Scheme 2. Synthesis of tested compounds (**60–62**).

phenyl-ethyl)-amide(22). Thionyl chloride (313 mg, 2.6 mmol) was added drop wise to a stirred solution of unsubstituted-1,8-naphthyridine-3-carboxylic acid (**21**, 500 mg, 2.2 mmol) in dichloromethane (30 mL) and catalytic amount of dimethyl formamide (2–4 drops). The stirring was continued for 4h at room temperature. The acyl chloride intermediate formed was dried under vacuum and again diluted with dichloromethane (30 mL). Functionalized amino acid (**3**) was added to it and stirred for 2h. The reaction mixture was diluted with water (30 mL) and extracted with dichloromethane (30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated to provide the crude product. The crude product was purified over silica gel (mesh size 100-200) column using 2% MeOH/ DCM as eluent, to furnish compound (**22**).

R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$ + DMSO-d_6) δ 10.62 (d, 1H, J=7.6 Hz), 9.14 (s, 1H), 8.86–8.81 (m, 2H), 7.62–7.44 (m, 4H), 7.33–7.19 (m, 2H), 5.88–5.86 (m, 1H), 5.65 (d, 1H, J=8.5 Hz), 5.39–5.33 (m, 3H), 4.32 (d, 1H, J=5.5 Hz), 2.73–2.72 (m, 1H), 2.66–2.61 (m, 1H, partially merged with DMSO peak), 0.63–0.59 (m, 2H), 0.43–0.41 (m, 1H), 0.34–0.33 (m, 1H); MS (ES+) 431 (M^++H), Yield 507 mg (53.6%), m.p. 152–154°C.

Compounds **23–59** were prepared in a similar to way to compound **22**.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide (23). R_f 0.5 (10 % MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.83 (d, 1H, J=8.2 Hz), 9.14 (s, 1H), 8.84–8.81 (m, 2H), 7.52–7.45 (m, 3H), 7.34–7.22 (m, 3H), 6.69 (d, 1H, J=7.6 Hz), 5.66 (dd, 1H, J=2.8, 8.2 Hz), 5.27 (s, 2H), 4.52–4.49 (m, 1H), 4.31–4.30 (m, 1H), 4.21–4.12 (m, 1H), 2.52 (t, 1H, J=2.2 Hz), 1.91–1.76 (m, 4H), 1.59–1.37 (m, 4H); MS (ES+) 459 (M^++H), Yield 850 mg (84.5%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclohexylcarbamoyl-2-hydroxy-1-phenyl-

ethyl)-amide **(24)**. R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.88 (d, 1H, *J*=8.4 Hz), 9.11 (s, 1H), 8.82 (d, 2H, *J*=6.5 Hz), 7.50–7.44 (m, 3H), 7.30–7.18 (m, 3H), 6.72 (d, 1H, *J*=8.5 Hz), 5.69 (dd, 1H, *J*=2.8, 8.4 Hz), 5.27 (d, 2H, *J*=2.4 Hz), 4.75 (d, 1H, *J*=4.5 Hz), 4.51 (bs, 1H), 3.76–3.73 (m, 1H), 2.52 (t, 1H, *J*=2.4 Hz), 2.10–2.09 (m, 1H), 1.83–1.49 (m, 5H), 1.27–1.08 (m, 4H); MS (ES+) 473 (M^++H) (100), Yield 810 mg (78.1%), m.p. 122–124°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide **(25)**. R_f 0.6 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.63 (d, 1H, *J*=8.8 Hz), 9.73 (s, 1H), 9.13 (s, 1H), 9.02–9.0 (m, 1H), 8.81 (d, 1H, *J*=6.5 Hz), 7.77–7.66 (m, 3H), 7.51–7.36 (m, 4H), 7.34–7.27 (m, 3H), 7.11–7.06 (m, 1H), 6.46 (d, 1H, *J*=5.7 Hz), 5.72 (d, 1H, *J*=8.8 Hz), 5.43 (s, 2H), 4.46 (d, 1H, *J*=5.7 Hz), 3.55 (1H, merged with water peak); MS (ES+) m/z 466 (M^++H), Yield 312 mg (30.5%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-(4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide **(26)**. R_f 0.6 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.54 (d, 1H, *J*=8.9 Hz), 9.78 (s, 1H), 9.05 (s, 1H), 8.94–8.92 (m, 1H), 8.72 (dd, 1H, *J*=1.7, 7.9 Hz), 7.68–6.61 (m, 3H), 7.42–7.24 (m, 5H), 7.09–7.03 (m, 2H), 6.38 (d, 1H, *J*=5.7 Hz), 5.63 (d, 1H, *J*=8.9 Hz), 5.36 (s, 2H), 4.39 (dd, 1H, *J*=2.1, 5.7 Hz), 3.48 (t, 1H, *J*=2.3 Hz); MS (ES+) m/z 485 (M^++H), Yield 325 mg (30.6%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-2-(4-methoxy-phenylcarbamoyl)-1-phenyl-ethyl]-amide **(27)**. R_f 0.4 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.90 (d, 1H, *J*=8.3 Hz), 9.09 (s, 1H), 8.80–8.76 (m, 2H), 8.67 (s, 1H), 7.49–7.39 (m, 5H), 7.30–7.16 (m, 3H), 6.73 (d, 2H, *J*=8.8 Hz), 5.81 (dd, 1H, *J*=2.3, 8.3 Hz), 5.22 (s, 2H), 5.13 (d, 1H, *J*=5.2 Hz), 4.69–4.66 (m, 1H), 3.71 (s, 3H), 2.50 (t, 1H, *J*=2.3 Hz); MS (ES+) m/z (relative intensity) 497 (M^++H), (100), Yield 510 mg (46.8%), m.p. 203–205°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-benzylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide **(28)**. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.56 (d, 1H, *J*=8.7 Hz), 9.1 (s, 1H), 8.97 (d, 1H, *J*=3.2 Hz), 8.70 (d, 1H, *J*=6.9 Hz), 8.33–8.31 (m, 1H), 7.69–7.65 (m, 1H), 7.41–7.25 (m, 5H), 7.06–7.05 (m, 2H), 6.88–6.87 (m, 3H), 6.24 (d, 1H, *J*=5.7 Hz), 5.62 (d, 1H, *J*=8.7 Hz), 5.52–5.37 (m, 2H), 4.50–4.42 (m, 1H), 4.28 (d, 1H, *J*=5.5 Hz), 4.10–4.04 (m, 1H), 3.54 (s, 1H); MS (ES+) m/z 481 (M^++H), Yield 716 mg (67.9%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide **(29)**. R_f 0.5 (EtOAc); ^{1}HNMR (DMSO-d_6) δ 10.61 (d, 1H, *J*=9.0 Hz), 9.7 (bs, 1H), 9.0 (s, 1H), 8.93 (d, 1H, *J*=4.1 Hz), 8.74 (d, 1H, *J*=7.4 Hz), 8.25 (d, 1H, *J*=3.6 Hz), 8.07 (d, 1H, *J*=8.4 Hz), 7.79–7.64 (m, 2H), 7.46–7.09 (m, 6H), 6.6 (d, 1H, *J*=5.4 Hz), 5.7 (d, 1H, *J*=8.8 Hz), 5.36 (s, 2H), 4.51 (d, 1H, *J*=5.4 Hz), 3.48 (s, 1H); MS (ES+) m/z 468 (M^++H), Yield 158 mg (15.4%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-3-ylcarbamoyl)-ethyl]-amide **(30)**. R_f 0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.94 (d, 1H, *J*=8.3 Hz), 9.12 (s, 1H), 8.92 (s, 1H), 8.83–8.78 (m, 2H), 8.40 (s, 1H), 8.21–8.17 (m, 2H), 7.51–7.46 (m, 3H), 7.34–7.23 (m, 4H), 5.80 (dd, 1H, *J*=2.3, 8.3 Hz), 5.73 (bs, 1H), 5.30–5.24 (m, 2H), 4.70 (d, 1H, *J*=2.3 Hz), 2.53 (t, 1H, *J*=2.4 Hz); MS (ES+) m/z 468 (M^++H), Yield 605 mg (58.9%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-4-ylcarbamoyl)-ethyl]-amide **(31)**. R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.92 (d, 1H, *J*=8.2 Hz), 9.13 (s, 1H), 8.96 (bs, 1H), 8.83–8.79 (m, 2H), 8.36–8.34 (m, 2H), 7.52–7.47 (m, 5H), 7.37–7.29 (m, 3H), 5.77 (d, 1H, *J*=5.7 Hz), 5.26 (d, 2H, *J*=2.1 Hz), 4.71 (d, 1H, *J*=2.7 Hz), 2.52 (t, 1H, *J*=2.4 Hz); MS (ES+) m/z 468 (M^++H), Yield 234 mg (22.8%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(thiazol-2-ylcarbamoyl)-ethyl]-amide **(32)**. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.84 (d, 1H, *J*=8.3 Hz), 10.69 (bs, 1H), 9.11 (s, 1H), 8.81–8.79 (m, 1H), 8.73–8.71 (m, 1H), 7.52–7.44 (m, 3H), 7.35–7.20 (m, 4H), 6.88 (d, 1H, *J*=3.5 Hz), 6.33 (bs, 1H), 5.86 (d, 1H, *J*=6.5 Hz). 5.24–5.21 (m, 2H), 4.80 (s, 1H), 2.51 (t, 1H, *J*=2.4 Hz); MS (ES+) m/z 474 (M^++H), Yield 578 mg (55.6%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-piperidin-1-yl-propyl)-amide **(33)**. R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.66 (d, 1H, *J*=8.8 Hz), 9.10 (s, 1H), 8.9–8.8 (m, 2H), 7.54–7.46 (m, 3H), 7.39–7.26 (m, 3H), 5.57 (d, 1H, *J*=8.2 Hz), 5.35–5.22 (m, 2H), 4.73 (d, 1H, *J*=4.8 Hz), 4.49 (d, 1H, *J*=6.4 Hz), 3.6-3.5 (m, 4H), 2.49 (t, 1H, *J*=2.4 Hz), 1.65–1.35 (m, 6H); MS (ES+) m/z 459 (M^++H), Yield 608 mg (60.4%), m.p. > 250°C.

4-Oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-pyrrolidin-1-yl-propyl)-amide **(34)**. R_f 0.4 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.72 (d, 1H, *J*=8.6 Hz), 9.11 (s, 1H), 8.85–8.81 (m, 2H), 7.52–7.46 (m, 3H), 7.38–7.26 (m, 3H), 5.61 (dd, 1H, *J*=2.5, 8.6 Hz), 5.26 (dd, 2H, *J*=2.3, 14.8 Hz), 4.54 (dd, 1H, *J*=2.5, 6.7 Hz), 4.28 (d, 1H, *J*=6.7 Hz), 3.57–3.42 (m, 4H), 2.5 (s, 1H), 1.97–1.85 (m, 4H); MS (ES+) m/z 445 (M^++H), Yield 292 mg (29.9%), m.p. 197-199°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide **(35)**. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.76 (d, 1H, *J*=7.8 Hz), 9.14 (s, 1H), 8.75 (d, 1H, *J*=8.2 Hz), 7.47–7.21 (m, 6H), 6.62 (d, 1H, *J*=7.3 Hz), 5.65 (d, 1H, *J*=7.0 Hz), 5.21 (s, 2H), 4.49 (s, 1H), 4.19–4.07 (m, 2H), 2.55 (s, 1H), 1.91–1.74 (m, 2H), 1.54–1.20 (m, 6H); MS (ES+) m/z 493 (M^++H), Yield 621 mg (66.3%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclohexylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide **(36)**. R_f 0.4 (5% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.76 (d, 1H, *J*=8.1 Hz), 9.11 (s, 1H), 8.74 (d, 1H, *J*=8.3 Hz), 7.46–7.23 (m, 6H), 6.57 (d, 1H, *J*=8.4 Hz), 5.65 (dd, 1H, *J*=2.6, 8.1 Hz), 5.20 (s, 2H), 4.50–4.48 (m, 1H), 4.16 (d, 1H, *J*=4.9 Hz), 3.77–3.70 (m, 1H), 2.55 (t, 1H, *J*=2.2 Hz), 1.84–1.81 (m, 1H), 1.55–1.51 (m, 2H), 1.37–0.88 (m, 7H); MS (ES+) m/z 507 (M^++H), Yield 598 mg (62.1%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide ***(37)***. R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.45 (d, 1H, J=8.8 Hz), 9.65 (s, 1H), 9.02 (s, 1H), 8.69 (d, 1H, J=8.3 Hz), 7.70 (d, 1H, J=8.3 Hz), 7.60 (d, 2H, J=7.8 Hz), 7.42–7.20 (m, 7H), 7.02–6.97 (m, 1H), 6.38 (d, 1H, J=5.5 Hz), 5.63 (d, 1H, J=8.1 Hz), 5.26 (s, 2H), 4.38 (d, 1H, J=3.8 Hz), 3.52 (s, 1H); MS (ES+) m/z 501 (M^++H), Yield 402 mg (42.2%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-(4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide ***(38)***. R_f 0.7 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.46 (d, 1H, J=9.0 Hz), 9.80 (bs, 1H), 9.04 (s, 1H), 8.71 (d, 1H, J=8.1 Hz), 7.73–7.64 (m, 3H), 7.40-7.25 (m, 5H), 7.09–7.07 (m, 2H), 6.41 (bs, 1H), 5.65–5.63 (m, 1H), 5.28 (s, 2H), 4.39 (bs, 1H), 3.54 (s, 1H); MS (ES+) m/z 519 (M^++H), Yield 208 mg (21.1%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-2-(4-methoxy-phenylcarbamoyl)-1-phenyl-ethyl]-amide ***(39)***. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.81 (d, 1H, J=7.9 Hz), 9.08 (s, 1H), 8.70 (d, 1H, J=8.3 Hz), 8.57 (s, 1H), 7.49–7.20 (m, 8H), 6.75 (d, 2H, J=8.7 Hz), 5.77 (d, 1H, J=7.9 Hz), 5.17 (s, 2H), 4.77 (d, 1H, J=5.2 Hz), 4.67 (s, 1H), 3.73 (s, 3H), 2.54 (s, 1H); MS (ES+) 531 (M^++H), Yield 446 mg (44.2%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-benzylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(40)***. R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.77 (d, 1H, J=8.4 Hz), 9.03 (s, 1H), 8.68 (d, 1H, J=8.3 Hz), 7.46–6.98 (m, 12H), 5.73 (dd, 1H, J=2.4, 8.4 Hz), 5.20 (s, 2H), 4.61–4.52 (m, 2H), 4.40 (d, 1H, J=5.3 Hz), 4.28–4.21 (m, 1H), 2.57 (t, 1H, J=2.4 Hz); MS (ES+) m/z 515 (M^++H), Yield 566 mg (57.8%), m.p. 140-142°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide ***(41)***. R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.50 (d, 1H, J=9.0 Hz), 9.68 (s, 1H), 9.02 (s, 1H), 8.71 (d, 1H, J=8.2 Hz), 8.24 (d, 1H, J=4.3 Hz), 8.05 (d, 1H, J=8.3 Hz), 7.78–7.69 (m, 2H), 7.43–7.21 (m, 5H), 7.10–7.06 (m, 1H), 6.57 (d, 1H, J=5.5 Hz), 5.67 (d, 1H, J=9.0 Hz), 5.26 (s, 2H), 4.48 (d, 1H, J=5.5 Hz), 3.52 (s, 1H); MS (ES+) m/z 502 (M^++H), Yield 281 mg (29.4%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-3-ylcarbamoyl)-ethyl]-amide ***(42)***. R_f 0.4 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.49 (d, 1H, J=8.9 Hz), 10.0 (s, 1H), 9.04 (s, 1H), 8.78 (bs 1H), 8.70 (d, 1H, J=8.3 Hz), 8.21 (d, 1H, J=4.0 Hz), 8.06–8.03 (m, 1H), 7.72 (d, 1H, J=8.3 Hz), 7.43–7.25 (m, 6H), 6.48 (d, 1H, J=5.7 Hz), 5.65 (dd, 1H, J=1.5, 8.9 Hz), 5.27 (s, 2H), 4.45–4.42 (m, 1H), 3.53 (t, 1H, J=2.3 Hz); MS (ES+) m/z 502 (M^++H), Yield 381 mg (39.9%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(thiazol-2-ylcarbamoyl)-ethyl]-amide ***(43)***. R_f 0.4 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.76 (d, 1H, J=8.2 Hz), 10.48 (bs, 1H), 9.1 (s, 1H), 8.67 (d, 1H, J=8.3 Hz), 7.50–7.22 (m, 7H), 6.89 (d, 1H, J=3.5 Hz), 5.99 (bs, 1H), 5.83–5.80 (m, 1H), 5.24–5.11 (m, 2H), 4.78 (s, 1H), 2.55 (t, 1H, J=2.3 Hz); MS (ES+) m/z 508 (M^++H), Yield 295 mg (30.6%), m.p. > 250°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-piperidin-1-yl-propyl)-amide ***(44)***. R_f 0.7 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.56 (d, 1H, J=9.0 Hz), 9.07 (s, 1H), 8.74 (d, 1H, J=8.3 Hz), 7.53–7.27 (m, 6H), 5.54 (d, 1H, J=9.0 Hz), 5.29–5.10 (m, 2H), 4.74–4.72 (m, 1H), 4.47 (d, 1H, J=6.4 Hz), 3.60–3.49 (m, 4H), 2.53 (t, 1H, J=2.5 Hz), 1.71–1.57 (m, 6H); MS (ES+) m/z 493 (M^++H), Yield 381 mg (40.6%), m.p. 180-182°C.

7-Chloro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-hydroxy-3-morpholin-4-yl-3-oxo-1-phenyl-propyl)-amide ***(45)***. R_f 0.6 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.37 (d, 1H, J=8.4 Hz), 9.06 (s, 1H), 8.71 (d, 1H, J=8.3 Hz), 7.69 (d, 1H, J=8.3 Hz), 7.41–7.24 (m, 5H), 5.42 (d, 1H, J=8.4 Hz), 5.30 (s, 2H), 5.20 (d, 1H, J=6.2 Hz), 4.76 (s, 1H), 3.50–3.40 (m, 9H); MS (ES+) m/z 495 (M^++H), Yield 276 mg (29.3%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid (2-cyclopropylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(46)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.62 (d, 1H, J=8.0 Hz), 9.12 (s, 1H), 8.51 (d, 1H, J=7.2 Hz), 7.44–7.26 (m, 5H), 6.79 (bs, 1H), 5.65–5.62 (m, 1H), 5.21 (s, 2H), 4.49–4.43 (m, 1H), 3.85 (bs, 1H), 2.69–2.57 (m, 2H), 0.74–0.65 (m, 2H), 0.48–0.33 (m, 2H); MS (ES+) m/z 483 (M^++H), Yield 328 mg (38.2%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(47)***. R_f 0.4 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.62 (d, 1H, J=8.1 Hz), 9.04 (s, 1H), 8.43 (d, 1H, J=7.2 Hz), 7.38–7.35 (m, 2H), 7.27–7.15 (m, 3H), 6.57 (d, 1H, J=7.6 Hz), 5.58 (d, 1H, J=6.0 Hz), 5.13 (s, 2H), 4.41 (s, 1H), 4.14–4.07 (m, 1H), 4.02 (d, 1H, J=4.8 Hz), 2.49 (s, 1H), 1.65–1.6 (m, 1H), 1.55–1.26 (m, 4H), 1.18–1.10 (m, 3H); MS (ES+) m/z 511 (M^++H), Yield 456 mg (50.2%), m.p. 214–216°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid (2-cyclohexylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(48)***. R_f 0.4 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.34 (d, 1H, J = 8.8 Hz), 9.07 (s, 1H), 8.65 (d, 1H, J = 7.7 Hz), 7.37–7.20 (m, 6H), 6.06 (d, 1H, J = 5.4 Hz), 5.48 (d, 1H, J = 8.8 Hz), 5.31 (d, 2H, J = 2.0 Hz), 4.16 (dd, 1H, J = 2.0, 5.4 Hz), 3.56–3.40 (m, 2H), 1.60–1.45 (m, 5H), 1.22–0.99 (m, 5H); MS (ES+) m/z 525 (M^++H), Yield 325 mg (34.8%), m.p. 215–217°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide ***(49)***. R_f 0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.42 (d, 1H, J=8.8 Hz), 9.63 (s, 1H), 9.06 (s, 1H), 8.64 (d, 1H, J=7.8 Hz), 7.61 (d, 2H, J=7.8 Hz), 7.44–7.23 (m, 7H), 7.04–7.02 (m, 1H), 6.39 (d, 1H, J=5.2 Hz), 5.65 (d, 1H, J=8.8 Hz), 5.29 (s, 2H), 4.40 (s, 1H), 3.54 (s, 1H); MS (ES+) m/z 519 (M^++H), Yield 301 mg (32.6%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-(4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide ***(50)***. R_f0.5 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.42 (d, 1H, J=8.5 Hz), 9.78 (s, 1H), 9.06 (s, 1H), 8.63 (m, 1H), 7.66–7.63 (m, 2H), 7.42–7.24 (m, 5H), 7.11–7.05 (m, 2H), 6.41 (d, 1H, J=5.4 Hz), 5.65–5.63 (m, 1H), 5.29 (s, 2H), 4.40 (s, 1H), 3.55 (s, 1H); MS (ES+) m/z 537 (M^++H), Yield 275 mg (28.8%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid [2-(3-chloro-4-fluoro-phenylcarbamoyl)-2-hydroxy-1-phenyl-ethyl]-amide ***(51)***. R_f0.7 (7% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.42 (d, 1H, J=9.0 Hz), 9.97 (s, 1H), 9.05 (s, 1H), 8.62 (d, 1H, J=7.7 Hz), 7.96–7.92 (m, 1H), 7.63–7.58 (m, 1H), 7.42–7.22 (m, 6H), 6.49 (d, 1H, J=5.7 Hz), 5.65–5.62 (m, 1H), 5.29–5.28 (m, 2H), 4.40 (dd, 1H, J=2.2, 5.7 Hz), 3.54 (t, 1H, J=2.3 Hz); MS (ES+) m/z 571 (M^++H), Yield 270 mg (26.6%), m.p. 202-204°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid (2-benzylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(52)***. R_f0.3 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.41 (d, 1H, J=8.6 Hz), 9.09 (s, 1H), 8.57 (d, 1H, J=7.8 Hz), 8.32 (s, 1H), 7.50–7.24 (m, 5H), 7.05–6.93 (m, 5H), 6.24 (s, 1H), 5.60–5.58 (m, 1H), 5.30 (s, 2H), 4.44–4.39 (m, 1H), 4.26 (s, 1H), 4.08–4.05 (m, 1H), 3.54 (s, 1H); MS (ES+) m/z 533 (M^++H), Yield 312 mg (32.9%), m.p.236–238°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide ***(53)***. R_f0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.46 (d, 1H, J=9.0 Hz), 9.67 (s, 1H), 9.07 (s, 1H), 8.67 (d, 1H, J=7.8 Hz), 8.25 (d, 1H, J=3.7 Hz), 8.04 (d, 1H, J=8.3 Hz), 7.79–7.38 (m, 1H), 7.43–7.22 (m, 5H), 7.11–7.07 (m, 1H), 6.60 (d, 1H, J=5.6 Hz), 5.68–5.65 (m, 1H), 5.28–5.27 (m, 2H), 4.48 (dd, 1H, J= 1.9, 5.6 Hz), 3.54 (t, 1H, J=2.3 Hz); MS (ES+) m/z 542 (M^++H), Yield 225 mg (23.3%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-3-ylcarbamoyl)-ethyl]-amide ***(54)***. R_f0.5 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.42 (d, 1H, J=8.6 Hz), 9.98 (s, 1H), 9.05 (s, 1H), 8.78 (bs, 1H), 8.62 (d, 1H, J=7.7 Hz), 8.21–8.22 (m, 1H), 8.05–8.02 (m, 1H), 7.42–7.24 (m, 6H), 6.48 (d, 1H, J=5.3 Hz), 5.64 (d, 1H, J=8.6 Hz), 5.27 (s, 2H), 4.42 (d, 1H, J=5.3 Hz), 3.54 (s, 1H); MS (ES+) m/z 520 (M^++H), Yield 298 mg (30.9%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-4-ylcarbamoyl)-ethyl]-amide ***(55)***. R_f0.6 (10% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.41 (d, 1H, J=9.0 Hz), 10.10 (s, 1H), 9.04 (s, 1H), 8.62 (d, 1H, J=7.7 Hz), 8.36 (d, 2H, J=5.7 Hz), 7.67 (d, 2H, J=6.2 Hz), 7.42–7.21 (m, 5H), 6.48 (d, 1H, J=5.7 Hz), 5.66–5.63 (m, 1H), 5.27 (d, 2H, J=1.8 Hz), 4.43 (dd, 1H, J=2.1, 5.7 Hz), 3.54 (s, 1H); MS (ES+) m/z 520 (M^++H), Yield 237 mg (25.6%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(thiazol-2-ylcarbamoyl)-ethyl]-amide ***(56)***. R_f0.5 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.61 (d, 1H, J=8.4 Hz), 9.03 (s, 1H), 8.38 (d, 1H, J=7.2 Hz), 7.41–7.15 (m, 5H), 7.01 (d, 1H, J=3.5 Hz), 6.80 (d, 1H, J=3.5 Hz), 6.27 (bs, 1H), 5.82–5.79 (m, 1H), 5.22-5.19 (m, 2H), 4.67 (s, 1H), 2.49–2.48 (m, 1H); MS (ES+) m/z 526 (M^++H), Yield 318 mg (34.0%), m.p. > 250°C.

7-Chloro-6-fluoro-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid (2-hydroxy-3-oxo-1-phenyl-3-piperidin-1-yl-propyl)-amide ***(57)***. R_f0.7 (10% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.47 (d, 1H, J=9.0 Hz), 9.06 (s, 1H), 8.50 (d, 1H, J=7.2 Hz), 7.51–7.24 (m, 5H), 5.54–5.51 (m, 1H), 5.24–5.08 (m, 2H), 4.73–4.72 (m, 1H), 4.48 (d, 1H, J=6.0 Hz), 3.61–3.49 (m, 4H), 2.55 (s, 1H), 1.98–1.99 (m, 1H), 1.78–1.44 (m, 5H); MS (ES+) m/z 511 (M^++H), Yield 377 mg (41.5%), m.p. 217-219°C.

7-Methyl-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid [2-hydroxy-1-phenyl-2-(pyridin-2-ylcarbamoyl)-ethyl]-amide ***(58)***. R_f0.3 (5% MeOH/DCM); ^{1}HNMR (DMSO-d_6) δ 10.62 (d, 1H, J=9.0 Hz), 9.67 (s, 1H), 8.97 (s, 1H), 8.59 (d, 1H, J=8.1 Hz), 8.24 (d, 1H, J=4.3 Hz), 8.04 (d, 1H, J=8.2 Hz), 7.78–7.73 (m, 1H), 7.51 (d, 1H, J=8.1 Hz), 7.43–7.21 (m, 5H), 7.09–7.05 (m, 1H), 6.56 (d, 1H, J=5.6 Hz), 5.67–5.64 (m, 1H), 5.33 (s, 2H), 4.48–4.47 (m, 1H), 3.48–3.47 (m, 1H), 2.65 (s, 3H); MS (ES+) m/z 504 (M^++H), Yield 457 mg (43.9%), m.p. > 250°C.

2-Hydroxy-3-[(4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carbonyl)-amino]-3-phenyl-propionic acid ethyl ester ***(59)***. R_f0.6 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 10.65 (d, 1H, J=8.6 Hz), 9.08 (s, 1H), 8.77–8.74 (m, 2H), 7.43–7.19 (m, 6H), 5.66–5.63 (m, 1H), 5.19 (dd, 2H, J=2.1, 7.4 Hz), 4.47 (d, 1H, J=2.1 Hz), 4.23-4.16 (m, 2H), 3.44 (d, 1H, J=5.2 Hz), 2.44 (s, 1H), 1.23 (t, 3H, J=7.1 Hz); MS (ES+) m/z 420 (M^++H), Yield 427 mg (46.4%), m.p. > 250°C.

6-Fluoro-4-oxo-1-prop-2-ynyl-7-pyrrolidin-1-yl-1,4-dihydro-[1,8]naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(60)***. To a solution of **47** (500 mg, 0.9 mmol) and triethylamine (290 mg, 2.8 mmol) in acetonitrile (20 mL) was added pyrrolidine (0.10 g, 1.4 mmol). The resulting mixture was refluxed for 3h, concentrated, diluted with water and extracted in dichloromethane (50 mL). The organic layer was dried over sodium sulphate and concentrated to dryness to afford a crude product, which was chromatographed on silica gel (mesh size 100-200) column with 2% MeOH/DCM as eluent to afford compound **60** (0.41 g, 76.9%).

R_f0.4 (8% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 11.09 (d, 1H, J=8.1 Hz), 8.81 (s, 1H), 8.03 (d, 1H, J=12.8 Hz), 7.47–7.22 (m, 5H), 6.71 (d, 1H, J=7.8 Hz), 5.61 (dd, 1H, J=2.6, 8.1 Hz), 5.05-5.04 (m, 2H), 4.50–4.49 (m, 1H), 4.19–4.16 (m, 1H), 3.81–3.80 (m, 4H), 2.45 (t, 1H, J=2.4 Hz), 2.03-1.24 (m, 12H); MS (ES+) m/z 546 (M^++H), Yield 408 mg (76.40%), m.p. > 250°C.

Compounds **61** and **62** were prepared in a similar way to compound **60**.

6-Fluoro-7-(3-methyl-piperidin-1-yl)-4-oxo-1-prop-2-ynyl-1,4-dihydro-1,8] naphthyridine-3-carboxylic acid (2-cyclopentylcarbamoyl-2-hydroxy-1-phenyl-ethyl)-amide ***(61)***. R_f0.5 (8% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 11.03 (d,

1H, *J*=8.1 Hz), 8.82 (s, 1H), 8.06 (d, 1H, *J*=13.7 Hz), 7.47–7.22 (m, 5H), 6.70 (d, 1H, *J*=7.8 Hz), 5.62 (dd, 1H, *J*=2.0, 7.8 Hz), 5.02 (s, 2H), 4.49–4.36 (m, 4H), 4.21–4.14 (m, 1H), 3.14–3.06 (m, 1H), 2.82–2.74 (m, 1H), 2.46–2.45 (m, 1H), 1.91–1.23 (m, 13H), 0.97 (d, 3H, *J*=6.5 Hz); MS (ES+) m/z 574 (M^++H), Yield 497 mg (88.6%), m.p. 177–179°C.

6-Fluoro-7-(3-methyl-piperidin-1-yl)-4-oxo-1-prop-2-ynyl-1,4-dihydro-[1,8] naphthyridine-3-carboxylic acid (2-hydroxy-1-phenyl-2-phenylcarbamoyl-ethyl)-amide **(62)**. R_f 0.5 (7% MeOH/DCM); ^{1}HNMR ($CDCl_3$) δ 11.15 (d, 1H, *J*=7.5 Hz), 8.80–8.73 (m, 2H), 8.05 (d, 1H, *J*=13.7 Hz), 7.55–7.49 (m, 4H), 7.34–7.04 (m, 6H), 5.72 (d, 1H, *J*=3.9 Hz), 5.24 (s, 1H), 4.99 (s 2H), 4.69 (s, 1H), 4.45–4.35 (m, 2H), 3.14–3.05 (m, 1H), 2.82–2.74 (m, 1H), 2.44 (s, 1H), 1.92–1.70 (m, 3H), 1.26–1.24 (m, 2H), 0.96 (d, 3H, *J*=6.2 Hz); MS (ES+) m/z 582 (M^++H), Yield 472 mg (84.3%), m.p. > 250°C.

Cytotoxicity

All the synthesized naphthyridine derivatives (**22–62**) were tested for *in vitro* cytotoxicity on eight tumors as well as on two non-tumorous cell lines and IC_{50} values were calculated in micro molar (μM). The human tumor cell lines used in the study are ovary (PA1), prostate (DU145), oral (KB), colon (SW620), breast (HBL100), lung (A-549), pancreas (MIAPaCa2) and leukemia (K562). All the 1,8-naphthyridine **22–62** and assay standard Doxorubicin HCl (data not shown) were also tested against normal mouse fibroblast (NIH3T3) and normal ovary (CHO) cell line to evaluate their cancer cell specificity (safety index). Derivatives of 1,8-naphthyridine **22-62** were screened for cytotoxic activity at the highest soluble concentration of 10 μM and on four lower concentrations on eight human tumor and two non-tumorous cell lines. Briefly, a three day MTT *in vitro* cytotoxicity assay was performed, which is based on the principle of uptake of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), a tetrazolium salt, by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product that is read spectrophotometrically [10]. MTT was dissolved in phosphate buffer saline with a pH of 7.4 to obtain an MTT concentration of 5 mg/mL; the resulting mixture was filtered through a 0.22-micron filter to sterilize and remove a small amount of insoluble residue. For each type of tumor and normal cell, 5000 to 10000 cells were seeded in a 96-well culture plate and incubated with various concentrations of 1,8-naphthyridine derivatives (**22–62**) in a CO_2 incubator for 72 h. Control cells not treated with 1,8-naphthyridine-3-carboxamide derivatives (**22–62**) were similarly incubated. The assay was terminated after 72 h by adding 125 μg (25 μL) MTT to each well, then incubating for 3 h, and finally adding 50 μL of 10% SDS-0.01N HCl to each well to lyse the cells and dissolve formazan. After incubating for 1 h, the plate was read spectrophotometrically at 540 nm and the cytotoxicity percentage calculated using the following formula: Cytotoxicity percentage = (1-(X/R_1)* 100, where X = (absorbance of treated sample at 540 nm)-(absorbance of blank at 540 nm), R_1 = absorbance of control sample at 540 nm)-(absorbance of blank at 540 nm). The cytotoxicity data is summarized in Table 3 and the compounds, which were inactive at 10 μM, are not listed.

Anti-inflammatory activity

1,8-Naphthyridine-3-carboxamide derivatives (22–62) were also able to down regulate the levels of LPS stimulated TNF-α, IL-1β, IP-10 and MIP-1-α secreted by DCs and identified to have potential anti-inflammatory activity. The down regulation of cytokine and chemokine levels by >25% was considered as significant.

BMDC based ex-vivo septic shock assay to evaluate potential anti-inflammatory activity: Primary DC cultures were generated from femoral bone marrow of 8–12 weeks old C57BL/6 mice [11]. Bone marrow progenitors were cultured in RPMI-1640 supplemented with 10% FBS (Hyclone) and rmGMCSF (20 ng/mL) at 37°C, 5% CO_2. Immature DCs were stimulated with lipopolysaccharide (LPS; 10 ng/mL,) and incubated with the naphthyridine carboxamide derivatives at various concentrations ranging from 0.001 to 10 μg/mL, preferably between 0.1 and 1 μg/mL for 24 h. The IL-1-β, TNF-α, MIP-1-α, and IP-10 secreted by the DCs were measured in culture supernatants by Enzyme Linked Immunosorbent Assays (R&D Systems Inc, MN, USA). Percentage change in cytokine/chemokine = {(B-A)/A*100, where B = concentration of cytokine/chemokine (pg/mL) secreted by LPS stimulated DCs when incubated with test molecule, A = concentration of cytokine/chemokine (pg/mL) secreted by LPS stimulated DCs alone. LPS treated DCs were used as positive control.

Results and discussion

1,8-Naphthyridine-3-carboxamide derivatives are divided into three categories based on the substitution pattern at C-6 and C-7 position, unsubstituted: compounds without any substitution at C-6 and C-7 position; monohalo substituted: C-7 chloro substituted; and dihalo substituted: C-6-fluoro-C-7-chloro substituted compounds.

In C-3' cycloalkyl derived unsubstituted 1,8-naphthyridine derivatives (**22–24**) were inactive on different cancer cell lines. While, monohalo substituted derivatives (**35** and **36**) have resulted in improved cytotoxicity compared to unsubstituted derivatives (**22–24**). The C-3' cyclohexyl substituted derivative **36** exhibited potent cytotoxicity on ovarian (PA-1) cancer cell line with IC_{50} of 1.19 μM and safety index of ~7 against normal ovary (CHO) and ~4 on NIH3T3 (normal fibroblast) cell lines. The dihalo-substituted 1,8-naphthyridine derivatives (**47–48**) showed potent to moderate cytotoxicity on ovarian and other cancer cell lines. The cyclopentyl-substituted derivative **47** has showed potent cytotoxicity with IC_{50} of 0.41, 0.77 and 1.5 μM on pancreas (MIAPaCa), leukemia (K-652) and lung (A549) cancer cell lines, respectively in this series. While, expansion of cycloalkyl ring from cyclopentyl (**47**) to cyclohexyl (**48**) leads to slight decrease in cytotoxicity.

In C-3' aryl substituted 1,8-naphthyridine derivatives (**25–27**), compound **25** has showed potent cytotoxicity

Table 3. *In vitro* cytotoxicity of 1,8-Naphthyridine derivatives (**22–62**).

		IC_{50} (μ_M)									
S. No.	Compound No	PA-1 (Ovary)	DU-145(Prostate)	KB (Oral)	SW620 (Colon)	HBL100 (Breast)	A549 (Lung)	MIAPaCa (Pancreas)	K-562 (Leukemia)	NIH3T3 (Normal fibroblast)	CHO (Normal ovary)
1.	Doxorubicin	0.63	0.10	3.0	0.08	0.24	0.08	0.15	0.10	0.39	1.0
2.	25*	9.1	2.9	>10	>10	5.9	6.09	9.81	>10	4.79	NA
3.	29**	0.41	>10	3.7	1.4	4.1	3.06	>10	4.4	2.2	NA
4.	35	3.12	>10	>10	6.2	8.99	>10	8.26	6.34	9.76	NA
5.	36	1.19	>10	>10	4.62	4.7	9	8	9.4	5	8.7
6.	38	1.2	6.1	2.6	3.2	6.9	>10	>10	>10	NA	NA
7.	39	3.22	>10	>10	5.27	8.8	>10	8.2	>10	7.3	NA
8.	40	8.1	1.6	>10	>10	>10	>10	>10	>10	NA	NA
9.	41	7.6	>10	>10	>10	>10	>10	9.0	>10	NA	NA
10.	42	2.0	>10	>10	7.8	7.5	>10	7.3	>10	5.5	NA
10.	43	3.49	>10	>10	7.13	4	9	7.6	9.2	1.8	9.7
11.	44	3.33	>10	>10	>10	>10	>10	>10	>10	NA	NA
12.	45	3.95	>10	>10	>10	9.1	>10	9.1	>10	NA	NA
13.	46	2.54	8.56	9.6	4.11	6.60	>10	>10	8.60	5.86	NA
14.	47	3.55	>10	>10	2.79	>10	1.5	0.41	0.77	1.05	NA
15.	48	1.7	>10	7.2	4.4	5.0	6.8	3.8	9,9	3.4	2.2
16.	49	>10	>10	>10	>10	4.33	4.82	1.28	2.50	2.24	NA
17.	51	3.6	6.1	3.5	2.8	8.2	9.9	6.7	>10	7.9	2.4
18.	52	>10	>10	>10	>10	9.26	6.99	3.17	5.39	2.85	NA
19.	53	2.62	3.45	9.12	3.79	3.17	9.53	2.83	8.20	4.86	NA
20.	54	3.1	>10	>10	7.9	8.7	>10	3.3	8.4	4.1	NA
21.	55	4.7	>10	8.42	3.35	5.5	>10	8.1	7	7.6	NA
22.	56	3.1	3.6	7.9	6.3	3.20	2.58	3.50	4.16	5.31	NA
23.	57	>10	>10	>10	>10	5.22	7.49	1.78	4.99	1.69	NA

** *Salt*

* *Precipitation observed during aqueous dilution*

Cytotoxicity was assessed by MTT assay as described in Methods. Data shown are IC_{50} of single independent experiments done in triplicates. If IC_{50} was not achieved even at the highest concentration tested i.e. 10 μM, it was represented as NA.

with IC_{50} of 2.9 μM on prostate (DU-145) cancer cell line. While, the other aryl substituted derivatives with electron withdrawing or donating groups (**26** and **27**) were inactive. Benzyl substituted derivative **28** has shown no activity. However, in mono halo substituted 1,8-naphthyridine derivatives, compound **38** with electron withdrawing group has showed potent cytotoxicity on oral (KB) cancer cell line with IC_{50} of 2.6 μM. *N'*-Benzyl substituted derivative **40** has resulted in selective potent cytotoxicity on prostate (DU145) cancer cell line with IC_{50} of 1.6 μM. In dihalo substituted 1,8-naphthyridine derivatives (**49–51**), compound **49** has showed good activity on pancreas (MIAPaCa) and leukemia (K562) cancer cell lines. Benzyl substituted derivative **52** has shown moderate cytotoxicity on pancreas cancer cell line.

In C-3' heteroaryl substituted 1,8-naphthyridine derivatives, amongst unsubstituted 1,8-naphthyridine derivatives (**29–32**), only compound **29** has showed potent cytotoxicity with IC_{50} of 0.41 and 1.4 μM on ovary (PA-1) and colon (SW620) cancer cell lines, respectively. Reversing the position of the nitrogen in pyridine ring from second position leads to complete loss of activity (**30** and **31**). In mono halo substituted 1,8-naphthyridine derivatives (**41–43**), improvement in cytotoxicity was observed in 3-amino pyridyl derivative (**42**) and thiazole (**43**) substituted derivatives but 2-amino pyridyl derivative (**41**) has showed very slight activity. In dihalo substituted 1,8-naphthyridine derivatives (**53–55**), compound **53** has showed broad spectrum of activity with IC_{50} < 4 μM on five cancer cell lines. While, compounds **54** and **55** have resulted in moderate cytotoxicity.

In C-3'-tertiary amine substituted 1,8-naphthyridine derivatives, unsubstituted compounds (**33** and **34**) were found to be inactive. Whereas, mono halo substituted derivatives piperidine (**44**) and morpholino (**45**) showed selective cytotoxicity on ovary (PA-1) cancer cell line. In dihalo substituted 1,8-naphthyridine derivative, compound **57** has shown potent activity on pancreas (MIAPaCa) cell line with IC_{50} 1.78 μM and modest to low activities on other cell lines. Further we have studied that substitution of C-7 position with group having inductive effect like methyl (**58**) leads to the complete loss of activity and replacement of the C-3' amide group by ester (**59**) linkage caused complete loss of activity.

As dihalo substituted 1,8-naphthyridine derivatives **47** and **49** have shown potent cytotoxicity, the C-7 chloro group of **47** and **49** was replaced with different secondary amine as shown in Scheme 2. But it leads to complete loss of activity (**60–62**). This indicates that C-7 halo group is essential for the activity.

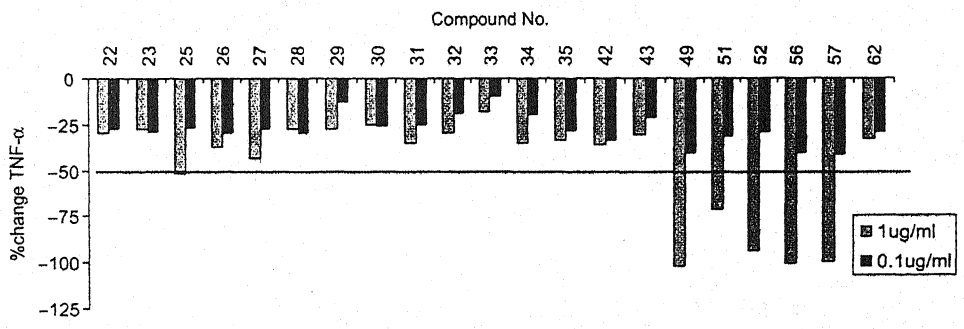


Figure 2. Anti-inflammatory activity as a measure of TNF-α downregulation.

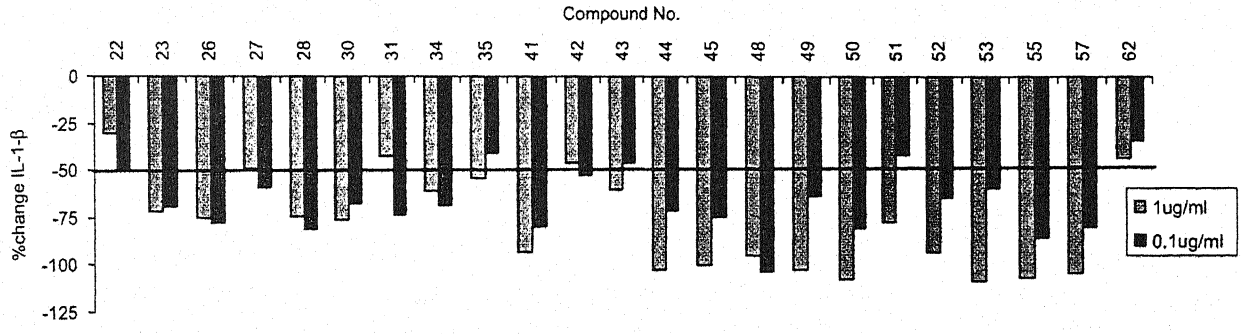


Figure 3. IL-1-β modulation of selected molecules. Dotted line shows IC_{50} (concentration at which 50% inhibition occurs).

The overall results indicated that halo substituted 1,8-naphthyridine derivatives with five and six membered cycloalkyl ring substituent have shown maximum cytotoxicity. Halo substituted compound **47** has shown IC_{50} of 0.41 and 0.77 μM on MIAPaCa and K-562 cancer cell lines, respectively. While, compound **36** has resulted in IC_{50} of 1.19 μM on PA-1 cancer cell line. However, one of the unsubstituted 1,8-naphthyridine 3'-heteroaryl derivative **29** has showed potent cytotoxicity with IC_{50} of 0.41 and 1.4 μM on ovary (PA-1) and colon (SW620) cancer cell lines, respectively. Replacement of C-7 halo group with secondary amine leads to loss of the activity.

Figure 2 shows the down regulation of TNF-α (primary mediator of tissue damage and pain in inflammatory disorders) by selected 1,8-naphthyridine-3-carboxamide derivatives. Compounds **49, 51, 52, 56** and **57** exhibited a very high TNF-α inhibition at 1 μg/mL. Table 4 demonstrates IC_{50} value for TNF-α inhibition by selected molecules screened at various concentrations ranging from 0.001 to 10 μg/mL.

Compounds showing high TNF-α down regulation **49, 51, 52** and **57** were also found to be potent inhibitors of IL-1-β secretion by LPS-stimulated DCs (Figure 3).

Inhibition of MIP-1-α and IP-10 (pro-inflammatory chemokines) activity is suggestive of anti-inflammatory activity of 1,8-naphthyridine-3-carboxamide derivatives. Compounds **44, 45, 49, 50, 53** and **55** showed >50% down regulation of MIP-1-α in addition to TNF-α and IL-1-β inhibition (Figure 4). Compounds **45, 48, 54, 55** and **56** have demonstrated high IP-10 inhibitory activity as shown in Table 5 & Figure 5.

Table 4. IC_{50} values for TNF-α modulation by selected 1,8-naphthyridine carboxamide derivatives. Molecules were subjected to screening over a multiple dose concentration range of 0.001 μg/mL to 10 μg /mL.

Molecule Numbers	IC_{50} value (μg)
41	<0.001
44	<0.001
45	0.31
48	<0.001
50	<0.001
53	<0.001
54	1.1
55	0.59

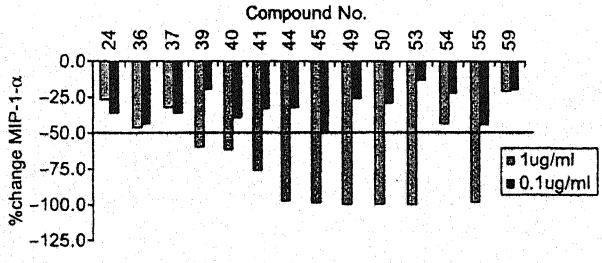


Figure 4. MIP-1-α modulation of selected molecules. Dotted line shows IC_{50} (concentration at which 50% inhibition occurs).

Compounds **45** and **55** were able to induce remarkable down regulation of TNF-α, IL-1-β, MIP-1-α and IP-10 activity and hence were found to be most active anti-inflammatory compounds among 1,8-naphthyridine-3-carboxamide derivatives.

Table 5. Downregulation of IP-10 levels to 50% (IC_{50}) of selected 1,8-naphthyridine derivatives. Molecules were subjected to screening over a multiple dose concentration range of 0.001 μg/mL to 10 μg/mL.

Molecule Number	IC_{50} value (μg)
55	0.62
45	0.32
54	1.1

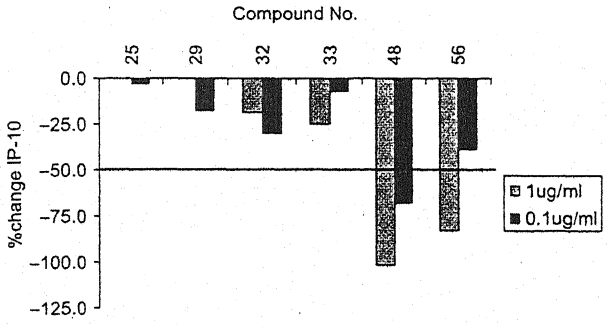


Figure 5. IP-10 modulation of selected molecules. Dotted line shows IC_{50} (concentration at which 50% inhibition occurs).

Acknowledgment

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Protection of phenyl glycine using BOC anhydride; Boc protection of an amino acid

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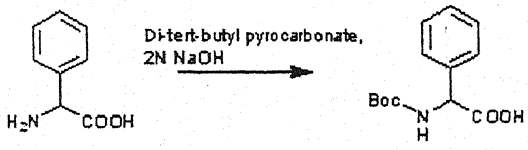

Chemicals Used

Phenyl glycine (SRL), Di-tert-butyl pyrocarbonate (Spectrochem), Sodium hydroxide (S.D. Fine), Hydrochloric acid (Rankem), Dioxane (Rankem), Ethyl acetate (Spectrochem)

Procedure

Phenyl glycine (10 g, 66.2 mmol) was dissolved with stirring in a mixture of dioxane (50 ml), water (25 ml) and 1N NaOH (10 ml). The reaction mixture was cooled using ice bath, and Di-tert-butyl pyrocarbonate (BOC anhydride, 15.8 g, 72.4 mmol) was added dropwise to the above solution. The cooled reaction mixture was stirred for 30 min, then reaction brought to rt and stirred for further 30 min. The solution was concentrated under vacuum to about 25-30 ml. The reaction mixture was again cooled using an ice bath, covered with a layer of ethyl

acetate (75 ml) and then acidified with a 1N HCl solution to pH 2-3. The aqueous layer was extracted with ethyl acetate (3 x 50 ml), the combined extracts washed with water (2 x 50 ml), dried over anhydrous sodium sulphate and concentrated under vacuum. The resulting crude product was chromatographed on silica gel (100-200 mesh size) column using 20% EtOAc/hexane as eluent to give the pure product as white solid (12 g, yield 72%).

Author's Comments

In this procedure 1N HCl has been used to extract the amino acid in ethylacetate in place of $KHSO_4$ as described in the literature.

Data

m.p. 88-90°C; Rf 0.5 (50% EtOAc/ Hexane); ^{1}HNMR ($CDCl_3$) 1.16-1.22 (m, 9H), 5.12 (d, 1H, J = 4.7 Hz), 7.29-7.43 (m, 5H), 8.03 (d, 1H, J = 4.2 Hz); MS (ES^+) m/z 274 (M+H+Na); HPLC purity = 99.2%

Lead Reference

The Practice of Peptide Synthesis, 2nd edition, M. Bodanszky, A. Bodanszky, 1994, page 17, Springer-Verlag Publications.

Other References

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Synthesis of Quinazolines as Tyrosine Kinase Inhibitors

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Abstract: In the present review, the discovery and development of quinazoline as tyrosine kinase inhibitors has been described. The synthesis of most potent quinazoline inhibitors of EGFR, VEGFR and PDGRF has been discussed. Structure activity relationship for quinazoline as tyrosine kinase inhibitors has been established. It was found that C-4, C-6 and C-7 positions in quinazoline are appropriate sites for designing new tyrosine kinase inhibitors. This review should help the medicinal chemist in designing more effective tyrosine kinase inhibitors.

1. INTRODUCTION

Although there has been progress in the development of therapy for cancer, this disease remains the second major cause of death in the US. Due to their general lack of specificity for tumors, drugs that are presently administered often lead to systemic toxicity and to undesirable side effects, such as hair loss and damage to the liver, kidneys, and bone marrow [1]. The most widely used anticancer drug, paclitaxel, was discovered in 1967 and was brought to the market as Taxol® in 1993 [2]. Since that time, small molecules such as imatinib (Gleevec), gefitinib (Irresa), erlotinib (Tarceva) and canertinib have been developed [3]. Still, the successful treatment of cancer remains a challenge in the 21st century, and there is a need to search for newer and safer anticancer agents that have a broader spectrum of cytotoxicity to tumor cells. In the last decade, there has been an increasing effort to discover new, small-molecule antitumor agents, with the main objective of targeting specific sites, especially those that are associated with the cell cycle [4]. Signaling pathways associated with growth factors, cytokines, hormones, and neurotransmitters are involved in the regulation of cellular functions; and the enzymes tyrosine kinase, thymidylate synthase, farnesyl protein transferase, c-SRC kinase, and aurora kinase are associated with the control of cellular signaling [5]. Tyrosine kinase, in particular, has been identified as a target for anticancer agents, and various quinazoline-based derivatives have shown activity against this enzyme. This review relates to the synthesis and biological activities of the most potent quinazoline derivatives.

2. TYROSINE KINASE INHIBITORS

In the mammalian signaling systems involving kinases, serine-threonine kinases and tyrosine kinases (TKs) are most common. The receptor tyrosine kinases promote signal transduction in tumor cells and endothelial cells by binding of the growth factors, epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF). For EGF, there are four closely related receptors: EGFR (ErbB-1), HER-2/neu (ErbB-2), HER-3 (ErbB-3) and HER-4 (ErbB-4). As EGFR inhibitors, certain anilinoquinazolines have good cellular activity and profound biochemical effects [6, 7]. VEGFR-2, also known as KDR, is a receptor tyrosine kinase expressed by endothelial cells, which are present in blood vessels. Various tumors use growth factors (including VEGF) to develop vascularization that allows them to grow beyond a small size [8]. Members of the PDGFR family, which include α-PDGFR, β-PDGFR, Flt-3, CSF-IR and c-Kit, are potent inducers of cell growth and mobility. Abnormal PDGFR-induced cell proliferation results in proliferative disorders, including cancer [9, 10]. In this review, the potent quinazoline inhibitors of EGFR, VEGFR and PDGFR are discussed.

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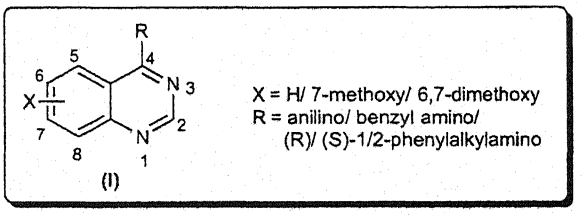
2.1. EGFR Inhibitors

Bridges *et al.* synthesized various quinazoline derivatives of prototype **I** as EGFR inhibitors [11]. Structure activity relationship has showed that compounds with dimethoxy substituents at C-6 and C-7 are found to be active. The 4-α-phenylethylaminoquinazoline with (*R*)-methyl substituent showed potent activity, while its (*S*)-enantiomer exhibited considerably weaker inhibitory activity. Increasing the side chain longer than methyl at benzylic CH is strongly disfavored. Simultaneously, increasing the size of the aromatic ring at C-4 from phenyl to naphthyl also diminished the cytotoxicity.

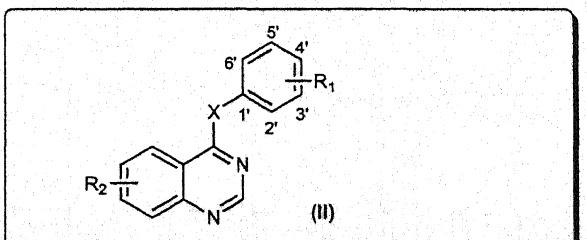


(I)

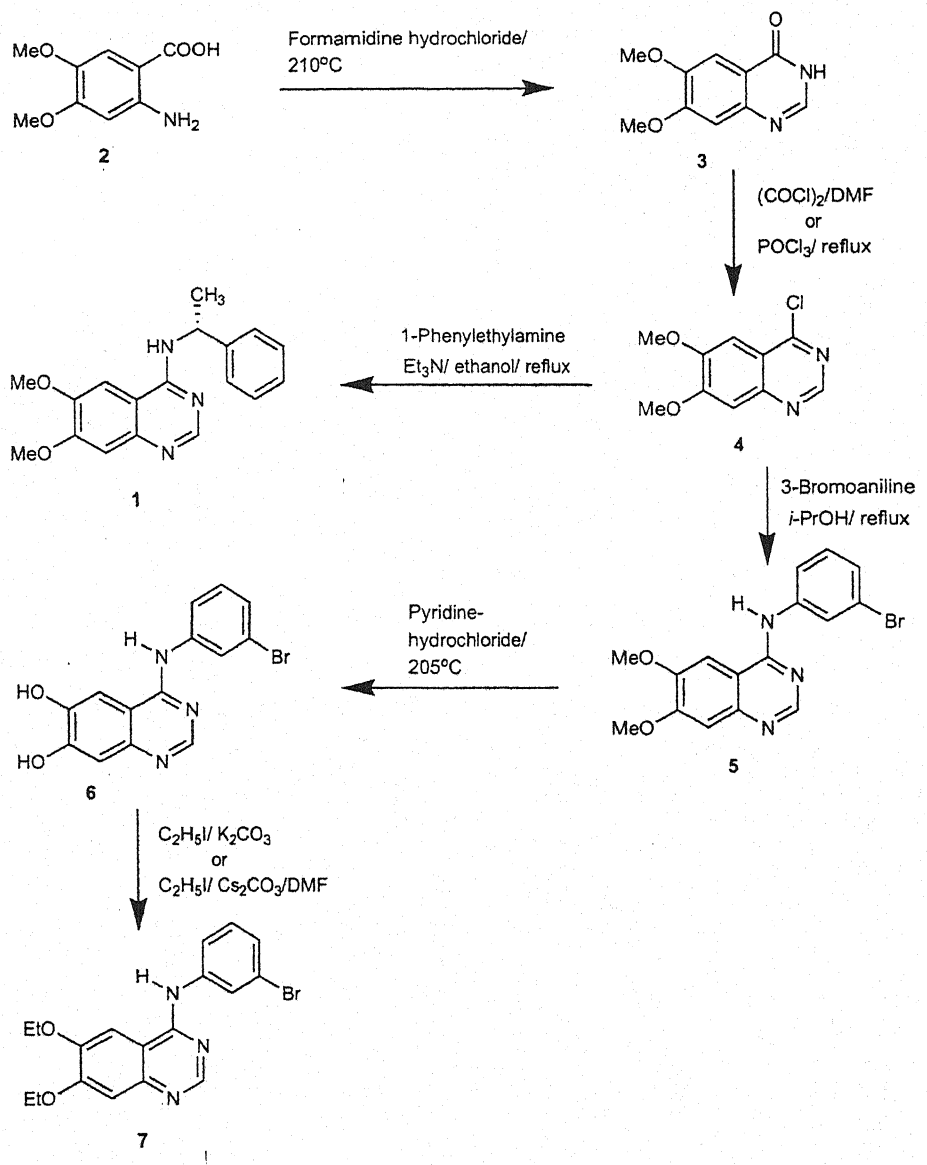
However, compound **1** was found to be most cytotoxic with IC_{50} of 1.6 nM for EGFR tyrosine kinase. Lineweaver-Burke analysis showed that compound **1** acts as a clean reversible ATP-competitive inhibitor with *Ki* of 627 pM. It was predicted that (*R*)-methyl group of compound **1** binds precisely in a small depression on the enzyme, while the (*S*)- methyl group causing unfavorable steric interactions with the other surface of the cleft.

Synthesis of the compound **1** is depicted in scheme **1** [11, 12]. The commercially available 6,7-dimethoxy anthranilic acid (**2**) was reacted with formamidine hydrochloride to furnish 6,7-dimethoxy quinazolone (**3**), which was converted into 4-chloro-6,7-dimethoxy quinazoline (**4**) by Vilsmeier procedure. Compound **4** was reacted with (*R*)(+)-phenylethylamine to provide compound **1**.

Rewcastle *et al.* synthesized a number of 4-substituted quinazolines of prototype **II** as inhibitor of tyrosine kinase activity of epidermal growth factor receptor [13].



(II)



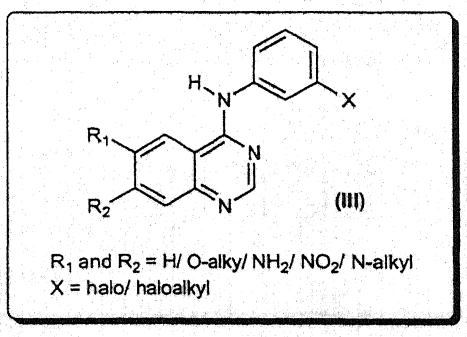
Scheme 1.

Structure activity relationship showed that the nature of the linking group between the quinazoline ring and phenyl side chain has the substantial effect on the inhibitory activity. Small lipophilic and electron withdrawing group at C-3' in phenyl ring has showed the potent cytotoxicity. It was interesting to note that alteration in the positions of nitrogen pattern in quinazoline ring leads to inactive compounds. Substitution of the quinazoline ring with electron donating group improved the potency.

Compound **5** is identified as a highly selective inhibitor of the tyrosine kinase and showed competitive inhibition with respect to ATP. Compound **5** has shown IC_{50} of 0.029 nM against the isolated enzyme of EGFR and IC_{50} of 15 nM for inhibition of EGF-stimulated tyrosine phosphorylation in NIH3T3 cells. Compound **5** was synthesized [13] by refluxing 4-chloro-6,7-dimethoxyquinazoline (**4**) with 3-bromoaniline in isopropanol, as shown in scheme **1**.

Based on the inhibitory properties of 4-anilinoquinazoline **5** against EGFR, as discussed above [14], Bridges *et al.* synthesized a number of new quinazoline derivatives of prototype **III** [12].

Structure activity relationship studies showed that, the compounds having halogen substituent at C-3 in aniline ring exhibited activity. Replacement of halogen group in aniline ring showed steric tolerance of the aniline-binding site. The supraadditive effect



was observed in 4-(3'-bromoanilino)quinazoline derivatives. It was also observed that those compounds, which are similar to compound **5**, did not show activity. So, only appropriately substituted quinazolines possess the ability to induce a change in the confirmation of the tyrosine kinase domain. The 6,7-ethoxy derivative **7** showed IC_{50} of 0.006 nM, while dimethoxy congener **5** with had shown IC_{50} of 0.025 nM against EGFR.

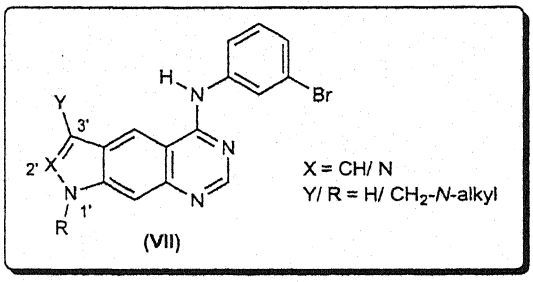
Compound **7** was synthesized starting from the compound **5** as in scheme **1** [12]. Demethylation of compound **5** was carried out in pyridine hydrochloride to give 6,7- dihydroxy derivative **6**, which on ethylation of compound **6** with ethyl iodide afforded the diethoxy compound **7**.

It was observed that, electron-donating substituents (like dimethoxy or hydroxy or amino groups) at C-6 and C-7 in quinazoline ring give rise to active compounds but the dimethoxy substituted quinazolines have shown relatively better activity profile. Based on these observations, Rewcastle *et al.* generated additional a 5- and 6- membered ring across C-6/ C-7 and C-5/C-6 positions. The fused tricyclic analogues of prototypes **IV**, **V** and **VI** were synthesized without increasing the bulk at C-5, C-6 and C-7 positions [15].

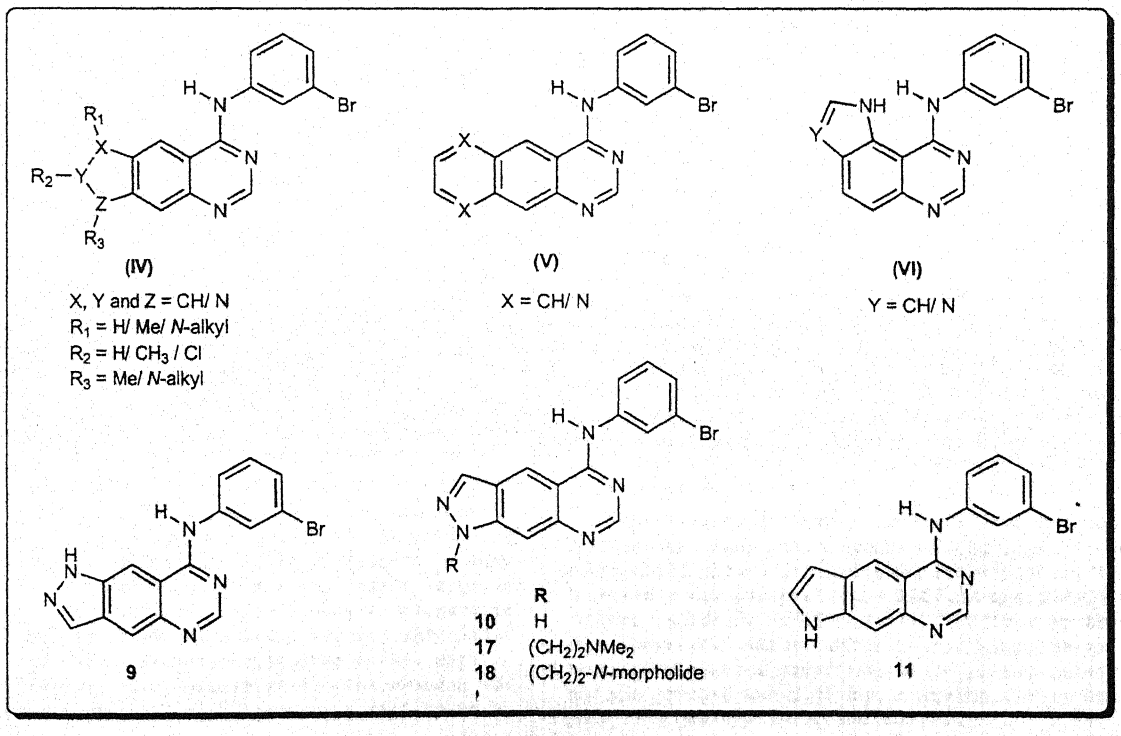
Structure activity relationship showed angular imidazoloquinazolines is less effective inhibitor than the linear isomers. Linear imidazolo-, pyrazolo- and pyrroloquinazoline compounds (**8-11**) have exhibited potent activity. Amongst them, linear imidazoloquinazoline **8** was found as most potent EGFR inhibitor. Compound **8** showed IC_{50} of 0.008 nM against phosphorylation caused by EGFR. Compound **8** is a potent inhibitor (IC_{50} of 46 nM) of autotophosphorylation of the EGFR in EGF-stimulated A431 cells. Compound **8** also exhibited similar activity against Swiss 3T3 cells. While compounds **9**, **10** and **11** showed IC_{50} of 0.34, 0.44 and 0.44 nM, respectively for EGFR inhibition.

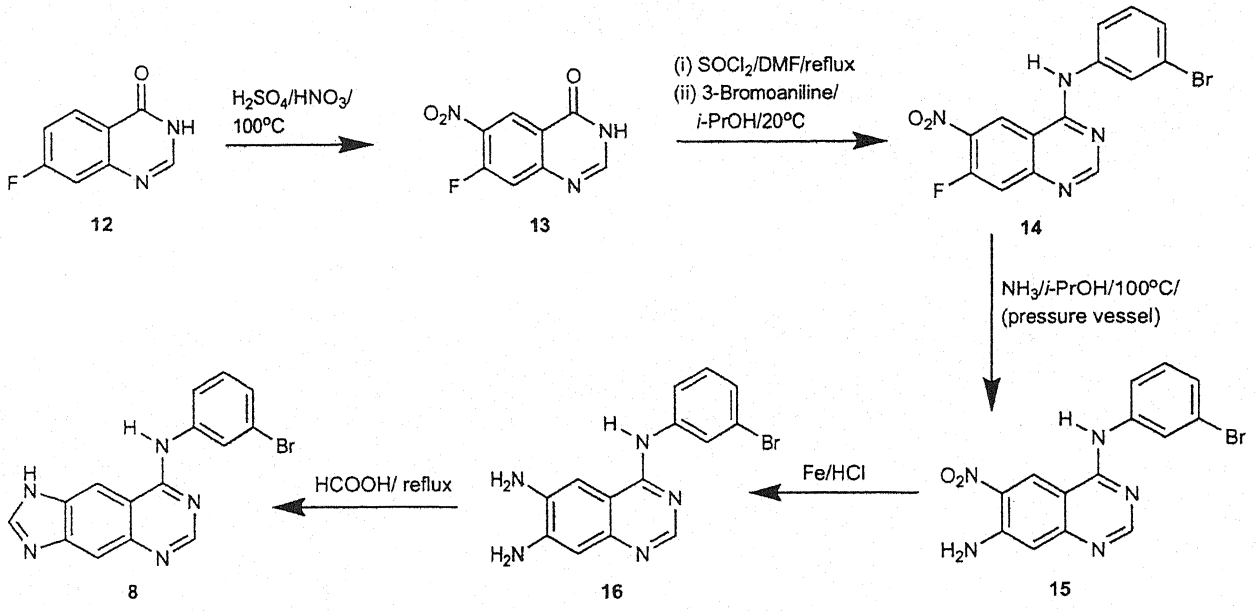
The imidazoloquinazoline derivative **8** was synthesized from 7-fluoroquinazolinone (**12**) as shown in scheme **2** [15]. Nitration of **12** with nitric acid yielded 7-fluoro-6-nitroquinazolinone (**13**). Compound **13**, upon treatment with thionyl chloride, yielded 4-chloroquinazoline derivative, which was reacted with 3-bromoaniline to provide 4-(3-bromophenyl) amino quinazoline (**14**). Reaction of **14** with ammonia afforded 7-amino 6-nitroquinazoline (**15**), which on reduction with iron and hydrochloric acid furnished the 6,7-diamino quinazoline compound **16**. The derivative **16** was refluxed with formic acid to give imidazoloquinazoline **8**.

Palmer *et al.* carried out the synthesis of various tricyclic quinazoline derivatives of prototype **VII** [16]. This study was based on the linear tricyclic quinazoline molecules **10** and **11** reported by Rewcastle *et al.* [15], which had shown poor aqueous solubility. Based on the molecular study and structure activity relationship, more soluble tricyclic analogues were synthesized.



Structure activity relationship studies showed that both nitrogen present in the quinazoline ring act as hydrogen bond acceptor and are essential for the activity. *N*-substituted pyrazoloquinazoline derivatives showed that H-bond donation is not critical. Highly basic side chains at *N*-1 enhanced the solubility (compound **17**), while the less basic side chain analogues are more active (compound **18**). Pyrroloquinazoline derivatives were, in general, more soluble than pyrazolo ones. Highly basic side chains in pyrroloquinazolines lead to loss of potency while the less basic chain (compound **19**) showed good activity. The C-3' substituted pyr-





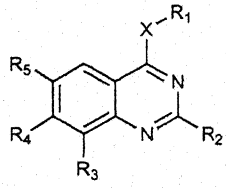
Scheme 2.

roloquinazoline derivatives (compounds **20-23**) were most effective with an average IC_{50} of 4 nM on EGFR. Compound **23** was found to be the most potent derivative with IC_{50} ~ 0.72 nM. These compounds were also evaluated for their ability to inhibit the autophosphorylation of EGFR in EGF stimulated A431 cells in culture. The pyrroloquinazoline derivative **19** was the most potent against the isolated enzyme with IC_{50} ~ 16 nM, while the parent compounds **10** and **11** showed IC_{50} ~ 20 nM. In general, it seemed that pyrroloquinazolines have shown better profile than pyrazoloquinazoline.

Active pyrroloquinazolines compounds (**19-23**) were synthesized from 3-methyl-4-nitrobenzoic acid (**24**) as depicted in scheme **3** [16, 17]. Compound **24** was converted into its ethyl ester by refluxing in ethanol in acidic medium to give **25**. The ethyl ester (**25**) was nitrated to give dinitro derivative **26**, which was condensed with DMF dimethyl acetal to give enamino ester **27**. Compound **27** was hydrogenated and cyclized to provide 6-amino-1-*H*-indole-5-carboxylic ethyl ester (**28**) by Batcho-Leimgruber indole synthesis. Compound **28** was treated with formamidine acetate in 2-methoxyethanol to provide 1,6-dihydro-5*H*-pyrrolo[3,2-*g*]quinazolin-5-one (**29**). Compound **29** was refluxed in $POCl_3$ to provide 4-chloro pyrroloquinazoline **30** and, which was treated with 3-bromoaniline to provide the compound **11** [15]. Compound **11**, upon treatment with chloroethyl-*N*-morpholide hydrochloride in presence of cesium carbonate, provided compound **19**. Compounds **20-23** were obtained on Mannich reaction of compound **11** with appropriate secondary amine in presence of acetic acid.

Myers *et al.* synthesized C-2/C-4/C-6/C-7/C-8 substituted quinazolines of prototype **VIII**, as EGFR and p56lck inhibitors [18]. The p56lck inhibitors are used in the treatment of autoimmune diseases, while EGFR inhibitors are related to anticancer activity [19].

Structure activity relationship for EGFR inhibitors showed that C-3'chloro substituent in phenyl ring at R_1 was found relatively better amongst other halo substituents. Upon replacement of nitrogen linker in 3'-chlorophenyl derived quinazolines by oxygen or sulfur causes small improvement in the activity but it reduced the activity in the compounds, which do not possess 3'-chlorophenyl group. The presence of electron donating group in phenyl ring at R_1 did not increase the activity. Quinazoline derivative possessing halo or alkyl substituents at C-2 and C-8 positions showed reduced activity, which indicates, and which showed the importance of steric hindrance in the N-1 interaction with the enzyme. However, compounds **31** and **32** were identified as most active EGFR inhibitors. Compounds **31** and **32** showed IC_{50} of 0.02 and 0.01 μM against EGFR autophosphorylation respectively.

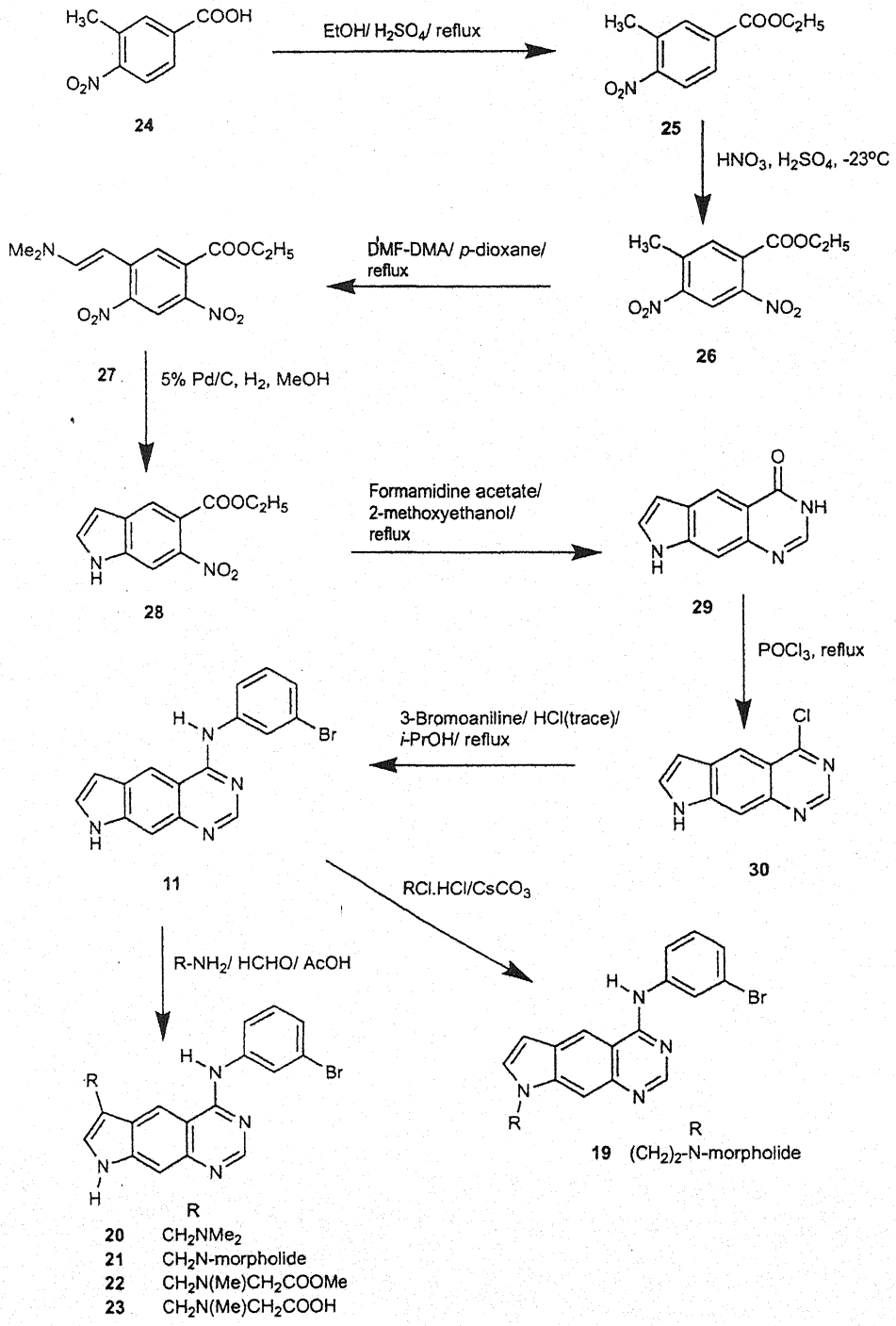


(VIII)

R_1 = aryl/ 3-haloaryl/ 4-hydroxyaryl/ 3,4-dimethoxyaryl/ 3,4,5-trimethoxyaryl
R_2 = H/ halo
R_3 = H/ alkyl/ alkoxy
R_4 = H/ alkoxy
R_5 = H/ halo/ alkoxy
X = O/ S/ NH

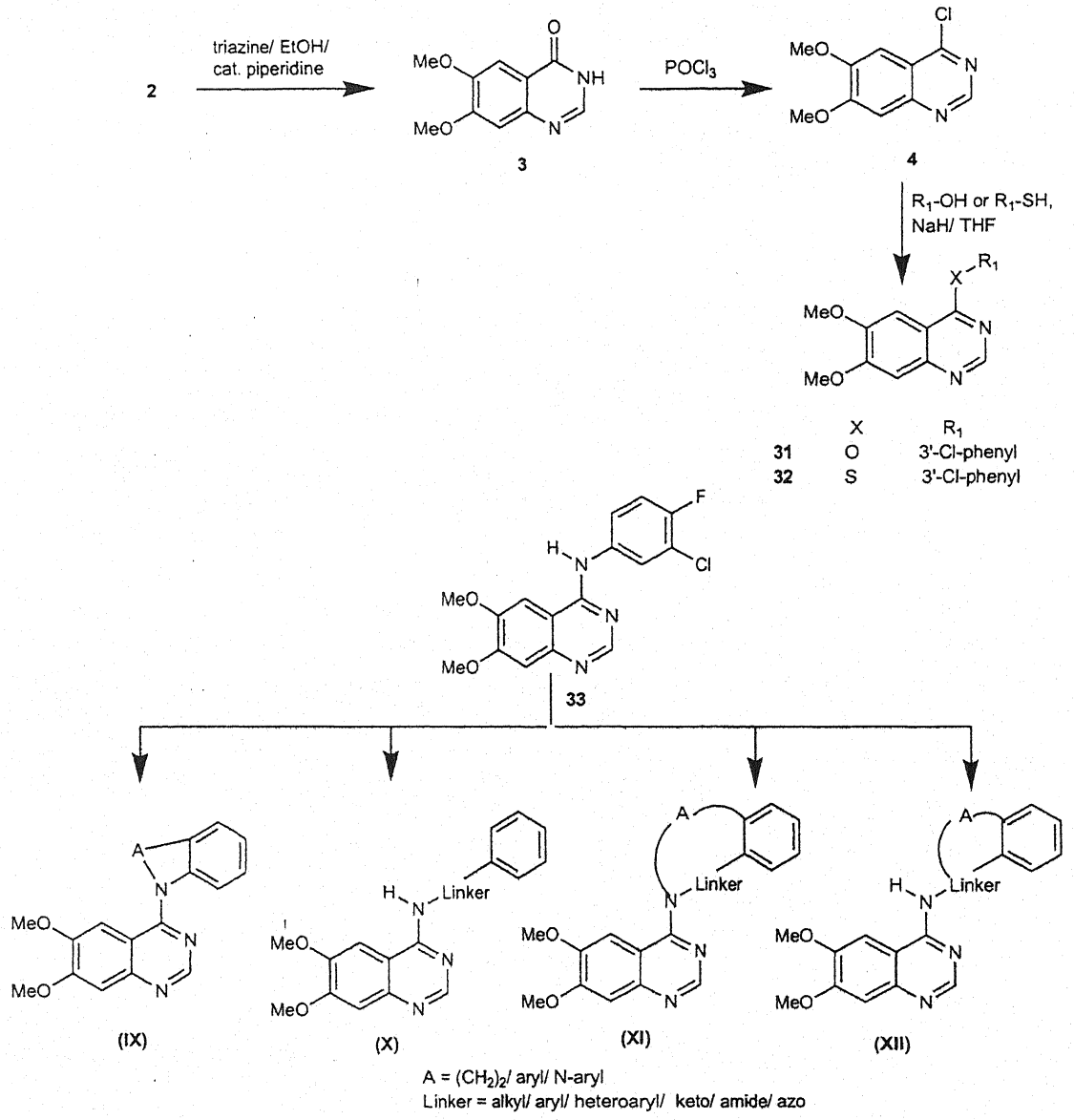
Compounds **31** and **32** were synthesized from 2-amino-4,5-dimethoxybenzoic acid (**2**) as in scheme **4** [18]. Compound **2** was cyclized to quinazolone **3** using triazine in presence of catalytic amount of piperidine. Compound **3** was converted to 4-chloroquinazoline **4** using phosphorus oxychloride. The 4-chloroquinazoline **4** was treated, with 3-chlorophenol and 3-chloro thiophenol to afford compounds **31** and **32**, respectively.

Till 1997, anilinoquinazoline had been well-recognized chemical class for having activity against the EGF-RTK enzyme and compound **33** has been identified as a potent inhibitor of EGF-RTK. It showed in vitro activity of IC_{50} ~ 0.009 μM against A431 cancer cell line and 0.08 μM on KB cancer cell line. Based on these findings, Gibson *et al.* had introduced different linking groups in between quinazoline and phenyl rings at C-4 position (prototype **X**) and also generated additional rings in between nitrogen linker and phenyl ring (prototype **IX**, **XI** and **XII**) [20].

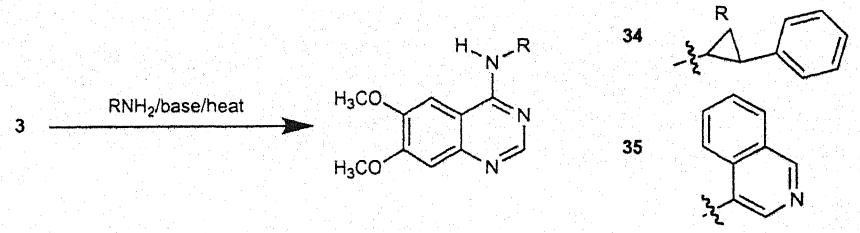


Scheme 3.

Structure activity relationship studies showed that modification in prototypes **X** and **XII** provided the most active derivatives such as compounds **34** and **35**. Compound **34** exhibited IC_{50} of 0.01 μM and 0.14 μM on EGF-RTK and KB cell lines, respectively but failed to show activity in tumor xenograft study. While, compound **35** showed IC_{50} of 0.0064 μM and 0.36 μM on the same cell lines, respectively. Further, modification at C-6 position in compound **34** provided compound **36**. Compound **36** is slightly less potent in comparison to **34** as IC_{50} values of **36** were found 0.027 μM and 0.31 μM on EGF-RTK and KB cell lines, respectively, but showed better activity in tumor xenograft study. Compounds **34** and **35** were synthesized from compound **3** as depicted in scheme **5** [20]. Compound **3** was treated, separately with 2-phenyl cyclopropylamine and isoquinolin-4-yl amine to provide compounds **34** and **35**, respectively.

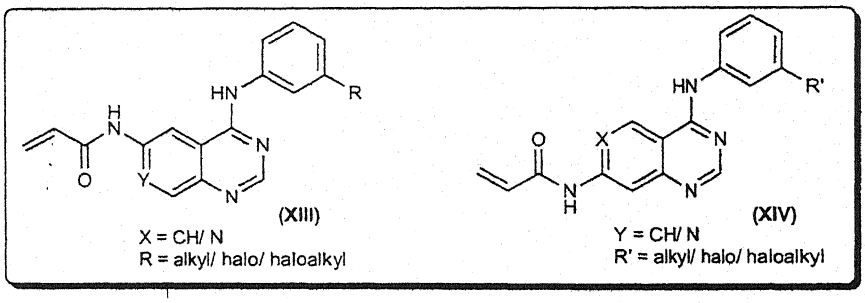


Scheme 4.



Scheme 5.

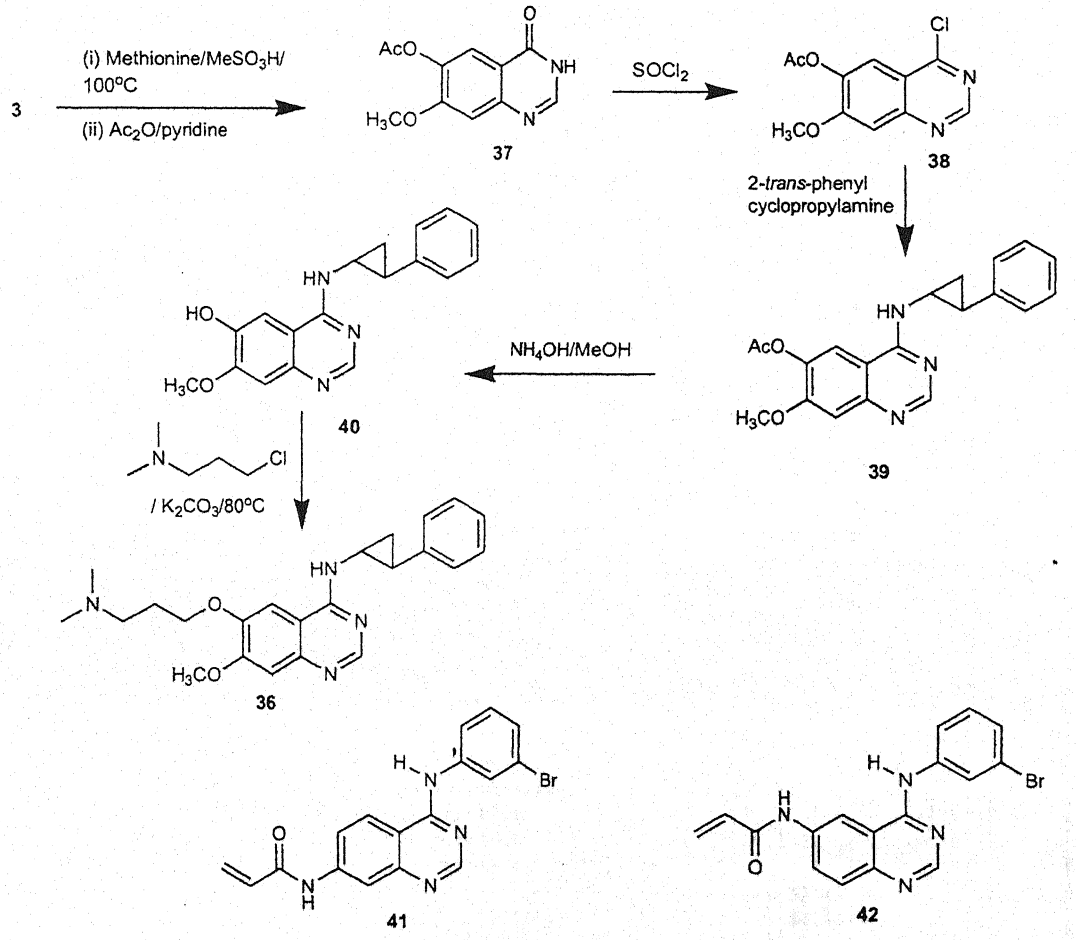
Compound **36** was synthesized starting from compound **3** as shown in scheme **6** [20]. Compound **3**, upon selective demethylation at C-6 position by methionine, yielded 6-hydroxy-7-methoxy quinazoline, which upon acetylation with acetic anhydride provided 6-acetyl-7-methoxy quinazolinone (**37**). Chlorination of **37** by thionyl chloride gave 4-chloroquinazoline **38**, which on reaction with *trans*-2-phenyl cyclopropylamine afforded 4-amino quinazoline derivative **39**. Deacetylation of compound **39** with ammonium hydroxide yielded 6-hydroxy quinazoline derivative **40**, which on the *O*-alkylation with (3-chloro-propyl)dimethylamine gave the desired compound **36**.



Smaill *et al.* carried out the synthesis and biological activity of C-6 and C-7-substituted quinazolines of prototype **XIII** and **XIV** [21]. The study was based on the 4-(phenylamino)quinazolines **41** and **42**, which are identified as irreversible inhibitors of EGFR [22]. The aim of study was to improve the solubility as well as antitumor activity.

Modeling data suggested that C-6 and C-7 positions could be substituted with bulky solubilizing functionalities. Acrylamido side chain (Michael acceptor) at C-6 position is optimally placed for reaction with Cys-773 in its most stable binding mode, whereas the acrylamido side chain at C-7 would require some movement in the binding site to allow it to approach close enough to the sulfur for Michael addition to occur, which is also proved by experimental results.

Structure activity relationship showed that changing the central chromophore of 7-acrylamide quinazoline (**41**) to pyridopyrimidine leads to the loss of property for irreversible inhibition, while the 6-acrylamide pyridopyrimidine retained the irreversible inhibition activity (compounds **45-47**). However, quinazoline derivatives **41**, **42**, **43** and **44** showed IC_{50} of 0.45, 0.70, 0.42 and 0.75 nM on EGFR isolated enzyme inhibition, respectively, whereas the pyridopyrimidine derivatives **45**, **46** and **47** showed IC_{50} of 0.91, 0.72 and 0.77 nM, respectively. These compounds were also tested for *in vivo* activity against the A431 epidermoid and H125 non-small-cell lung cancer human tumor xenograft models. *In vivo* studies showed significant tumor growth inhibition and acceptable therapeutic indices. The net tumor cell kill values were around −0.5 to +0.5 log unit.



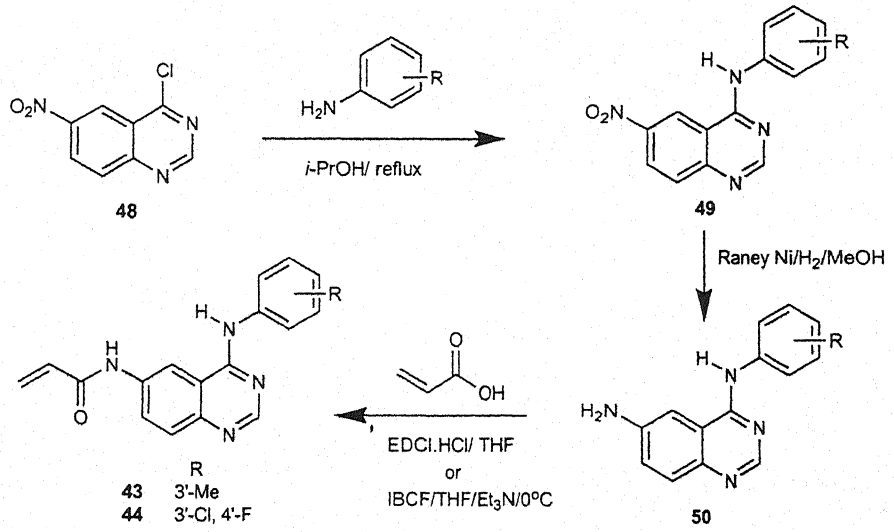
Scheme 6.

Quinazoline derivatives **43** and **44** were synthesized from 4-chloro-6-nitroquinazoline (**48**) [22, 23] as shown in scheme 7 [21]. Compound **48** was reacted with substituted aniline to provide compound **49**. The nitro group of **49** was reduced to the corresponding amino derivative **50**. The amino group of compound **50** was reacted with acrylic acid using EDCI or IBCF to provide the desired molecules.

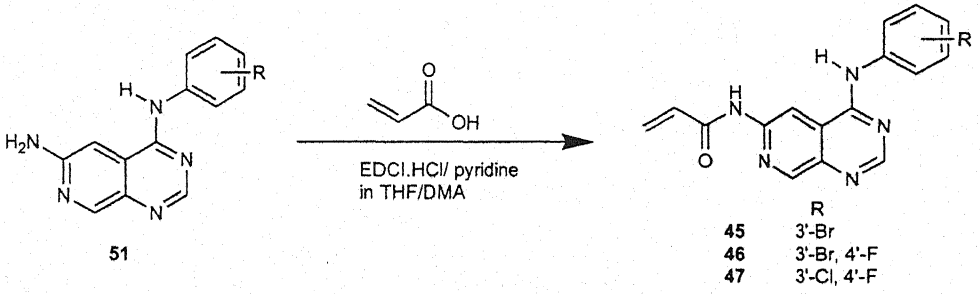
Pyridopyrimidines were synthesized from 4-anilno- 6-aminopyrido[3,4-*d*]pyrimidine (**51**) as depicted in scheme **8** [24, 25]. Compound **51** was coupled to acrylic acid as discussed for **43** and **44**, to produce compounds **45-47**.

Smaill *et al.* synthesized acrylamide-substituted 4-anilinoquinazolines and 4-anilino[*d*]pyrimidines derivatives of prototype **XV** [26]. This prototype is related to compounds **52** and **53**, which are selectively potent irreversible inhibitors of tyrosine kinase of *erb*B family [21]. Substitution was carried out at C-7 position with solubilizing cationic side chains (alkylamine and alkoxyamine).

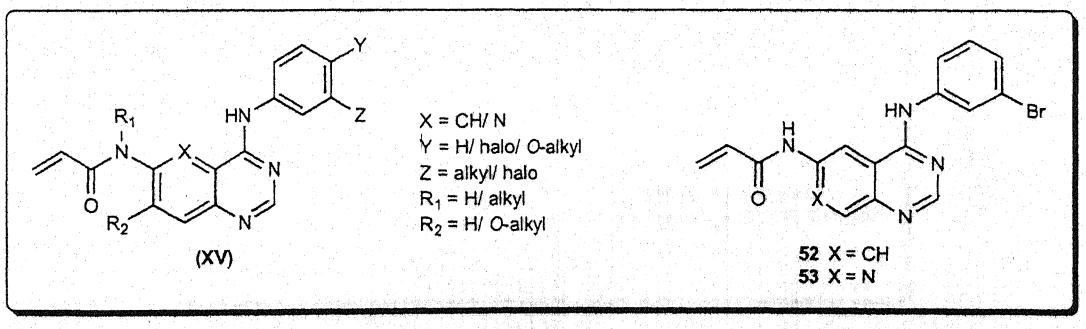
These compounds showed better aqueous solubility, potency and *in vivo* antitumor activity. Substitution of C-4' of the aniline ring with cationic side chain caused loss of binding affinity in ATP binding domain of EGFR. The acrylamide analogues with solubilizing cationic side chain provided the irreversible inhibitors. While, the cationic unsaturated side chain at C-7 position in case of



Scheme 7.



Scheme 8.



pyrido[3,2-*d*]-pyrimidines provided highly potent compound but these compounds showed several problems related to metabolism, transport, permeability and stability. Based on this study, a series of "oxygen linked" quinazoline (compound **54**) was synthesized. Compound **54** (CI-1033) exhibited IC_{50} of 1.5 nM in isolated EGFR enzyme inhibition and IC_{50} of 7.4 and 9.0 nM in autophosphorylation assay against EGFR and *erb*B2, respectively. Compound **54** also showed superior *in vivo* antitumor activities, when compared to earlier nonsolubilized analogues.

Compound **54** was synthesized from (3-chloro-4-fluoro-phenyl)-(7-fluoro-6-nitro-quinazolin-4-yl)-amine (**55**) [15] as shown in scheme **9** [26]. The fluorine group was replaced by 3-morpholin-4-yl-propan-1-ol to yield compound **56**. The nitro group of **56** was reduced to amino group to furnish compound **57**, which was coupled with acrylic acid to afford the desired compound **54**.

As discussed above, CI-1033 (compound **54**), having 6-acrylamide group, is an irreversible inhibitor of both EGFR and erbB2 autophosphorylation and showed more activity in comparison to reversible inhibitors.

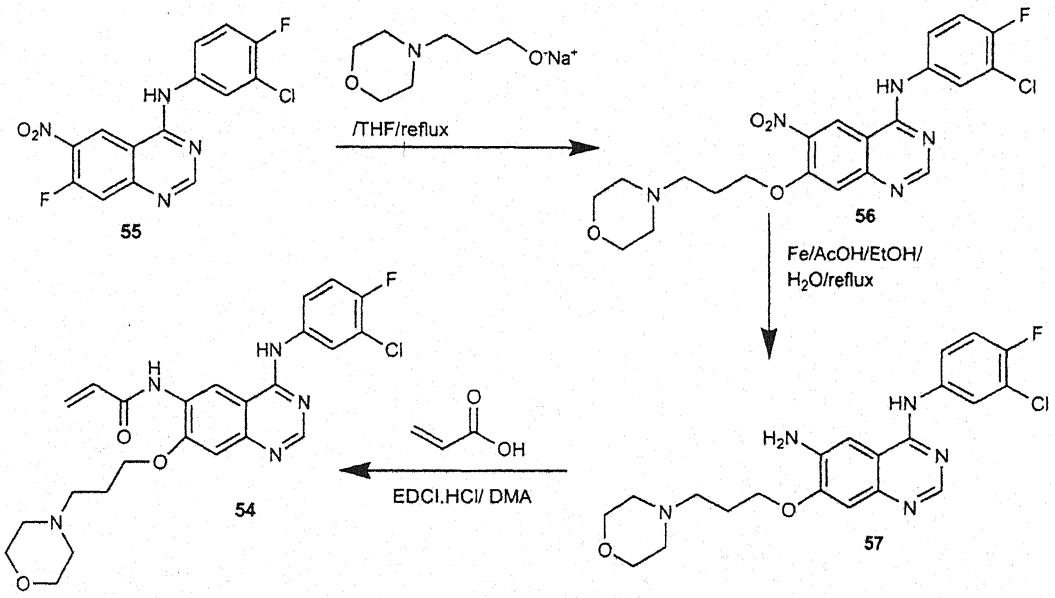
Based on this study, Smaill *et al.* synthesized several 4-anilinoquinazoline and 4-anilnopyrido[3,4-*d*]pyrimidine analogues of prototype **XVI** [27], in which different substituted Michael acceptor were placed at C-6 position with different solubility enhancing group.

Structure activity relationship studies showed that *N*-methyl at C-6 position (**59**) possessed irreversible inhibition with high potency while the larger group decreased the activity. Substitution at α-allylic position is not tolerable. Substitution at β-position in acrylamide by electron withdrawing groups increase the electrophilicity of the double bond of the Michael acceptor and resulted in fully irreversible compounds but a balance is required in reactivity of Michael acceptor and steric bulk at this position. A little difference of activity was observed in between the quinazoline and pyrido[3,4-*d*]-pyrimidine chromophores. Compounds bearing non-acrylamide Michael acceptor at C-6 position (like sulfonamide) showed potent and irreversible inhibition of EGFR but these compounds were unstable in the biological system.

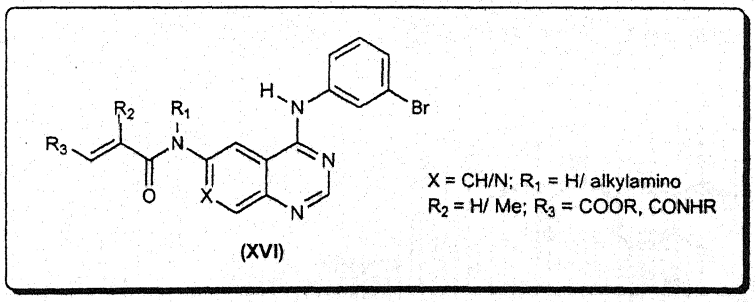
Compounds **59** and **64** were found most active with IC_{50} of 0.17 nM and 0.76 nM against the EGFR enzyme, respectively. But both the compounds were ineffective against the A431, H125 and MCF-7 xenografts.

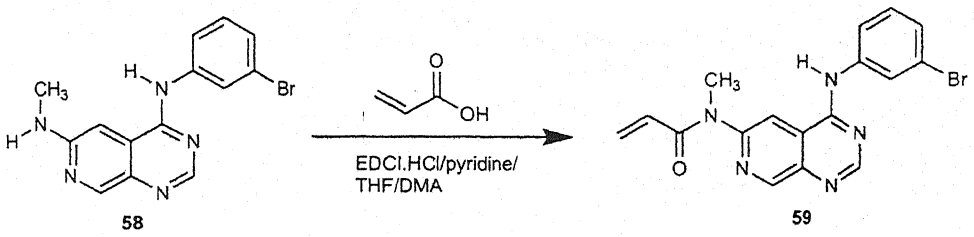
The coupling of 6-aminomethyl pyrido-pyridinyl derivative (**58**) [24] with acrylic acid furnished the compound **59** as depicted in scheme **10** [27].

Compound **64** was synthesized from (*E*)-but-2-enedioic acid monoethyl ester (**60**) as shown in scheme **11**. Compound **60** was chlorinated with oxalyl chloride, followed by amine to furnish amide ester (**61**). The ethyl ester group of **61** was hydrolyzed to acid



Scheme 9.





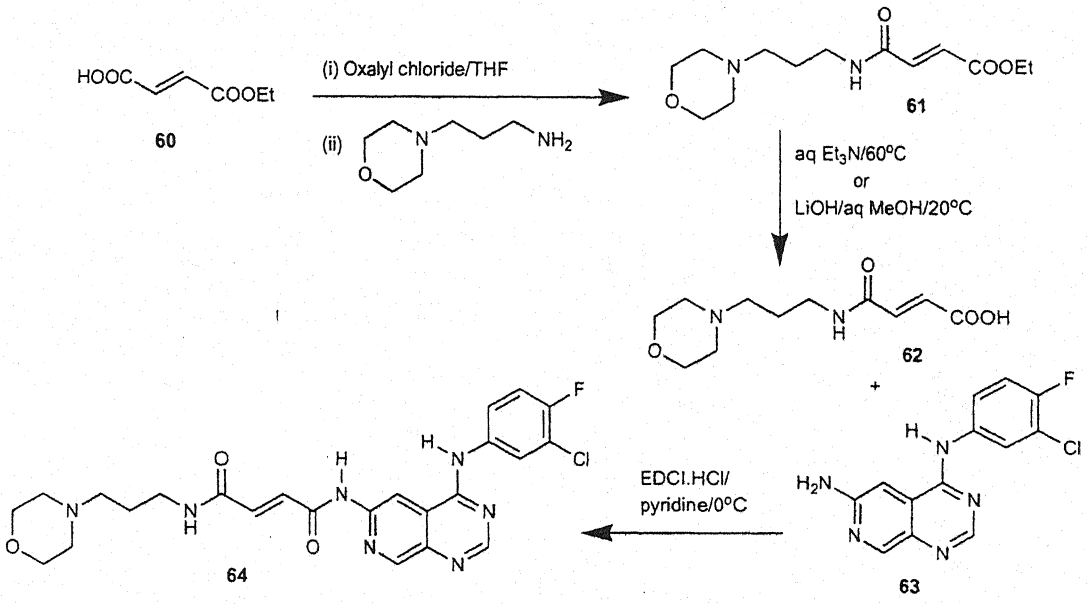
58 59

Scheme 10.

62, which was coupled with 6-amino pyrido-pyridinyl chromophore (63) [21] to afford compound 64.

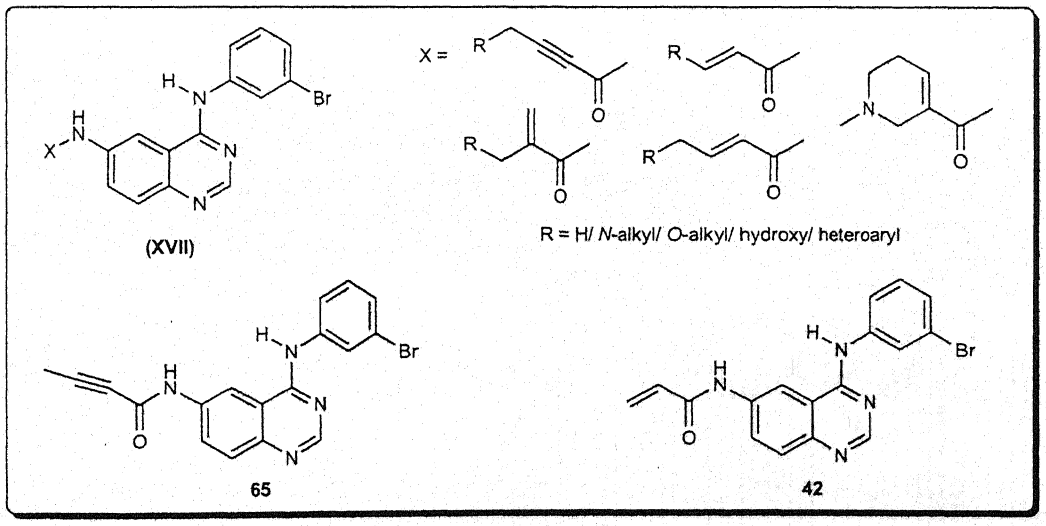
Based on the 4-(anilino)quinazoline core structure, irreversible inhibitors of epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor (HER-2), various derivatives of prototype XVII have been synthesized by the Tsou *et al.* [28]. The designing of the derivatives was based on the two earlier active compounds 65 and 42 [29, 30]. These compounds (65 and 42) exhibit poor bioavailibility due to low solubility under physiological condition.

In order to improve the water solubility, the water solubilzing groups (dialkylamino) were attached directly on the Michael accep-



60 61 62 63 64

Scheme 11.



(XVII) 65 42

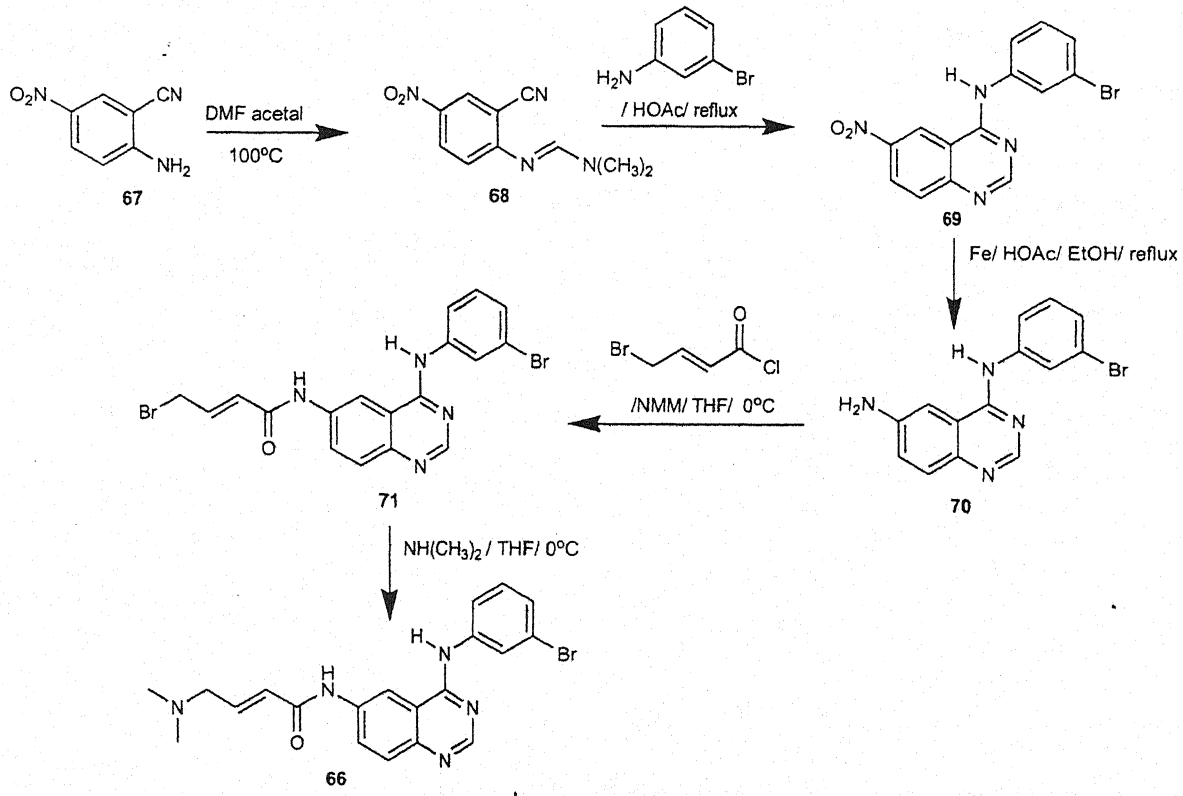
tor (crotonamide group), which is available at position C-6. Dialkylamino group exists as protonated form and exerts the inductive effect on Michael addition of sulfhydryl group of Cys 773. Compound **66**, showed better oral antitumor activity in comparison to compounds **65** and **42**.

Compound **66** is highly potent in inhibiting EGFR and HER-2 kinases receptors with IC_{50} of 0.011 μM and 0.301 μM, respectively. However, compound **66** was less potent in inhibiting KDR, EcK, Mek/ErK, PDFGR and VEGF kinases as IC_{50} value was found > 40 μM.

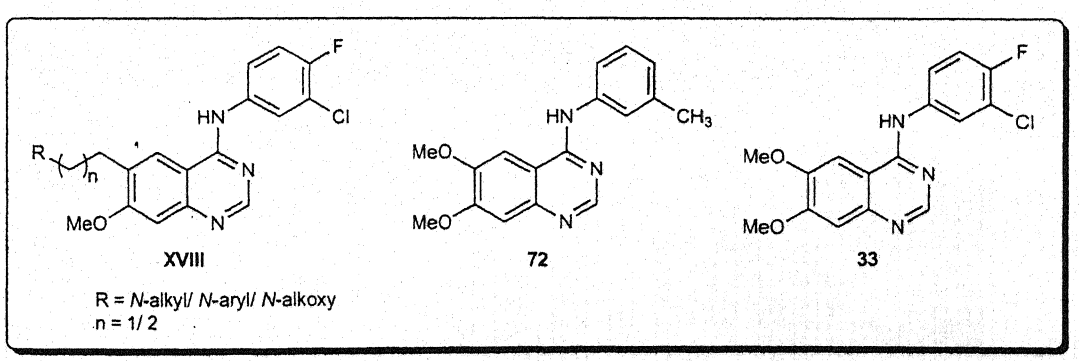
The synthesis of compound **66** was described from 5-nitroanthranilonitrile (**67**) in scheme **12** [28]. Compound **67** was converted with DMF-dimethylacetal into corresponding formamidine (**68**), which on treatment with 3-bromoaniline in acetic acid afforded 6-nitro-4-(3-bromophenylamino)quinazoline (**69**). The reduction of the nitro group of **69** with iron in acetic acid provided 6-aminoquinazoline (**70**), which on condensation with 4-bromocrotonyl chloride yielded **71**. Nucleophilic displacement of bromo group in **71** by *N,N'*-dimethylamine provided compound **66**.

Compound **72** is a potent inhibitor of EGFR-TK [31] but undergoes rapid metabolism on oral dosing [13]. To increase its stability, methyl group was replaced by the chloro and fluoro at C-3' and C-4', respectively (compound **33**). Although potency of compound **33** was decreased in comparison to compound **72** but it showed better *in vivo* profile and improved efficacy. Barker *et al.* carried further attempts to increase *in vivo* activity by modifying the methoxy group in compound **33** and synthesized the compounds of prototype **XVIII** [32].

Structure activity relationship studies showed that morpholino derivatives possessed high blood concentration after 6h and also sustained at 24h but the compound with 2-hydroxy substituent at C-6 position showed less blood concentration. Compound **73** was identified as a most potent enzyme inhibitor with IC_{50} value of 2 nM.



Scheme 12.



In EGF-stimulated cell proliferation test, compound **74** was found as a most potent inhibitor of tumor cell growth with IC_{50} of 80 nM. Compound **74** inhibits the growth of a broad range of human solid tumor xenografts, which was later launched in the market as Gefitinib (Irresa).

Compounds **73** and **74** (Irresa/ ZD1839) were synthesized from compound **38** as depicted in scheme **13** [32]. Compound **38** was reacted with 3-chloro-4-fluoroaniline to afford compound **75**. The acetyl group was now converted to hydroxy group (**76**) with aqueous methanolic ammonia. Hydroxy group was alkylated using two different routes. In the first synthetic route, hydroxy group was directly alkylated with side chain in one step. In second method, bromoalkyl group was introduced and bromo was displaced with appropriate amine to afford compounds **73** and **74**.

Cockreill *et al.* has found that compound **78** (4557W) acts as a potent inhibitor of c-erb-2 and EGFr [33]. The binding hypothesis has shown that benzyloxyaniline group, accommodated in the back of hydrophobic pocket with 6,7-dimethoxy groups, is pointing towards the tip of ATP binding cleft. A conformational restriction was carried out at the aniline fragment by placing different bicyclic ring (such as substituted indole, indazole, imidazole and benzotriazole) in the quinazoline and pyrido[3,4-*d*] pyrimidine ring systems of prototype **XIX**, to study the effect of hydrophobic binding interaction [34].

The compounds **79**, **80** and **81** were synthesized and evaluated for their anticancer activity and showed IC_{50} of 0.01, 0.001 and 0.027 μM, respectively against c-erB-2 inhibition (enzyme potency). Amongst them, compound **79** (GW974) showed the selectivity for EGFR subfamily. Compound **81** also exhibited the activity against CaLu3, HN5 and BT474 cells. Compound **81** showed 24% bioavailability in comparison to 7% of corresponding quinazoline analogue **80**.

The synthesis of pyridopyrimidine **81** starting from 6-chloronicotinic acid (**82**) is depicted in scheme **14** [34]. Curtius rearrangement and ortholithation of Boc-amine (**83**) afforded anthranilic acid derivative (**84**), which was cyclized to pyridopyrimidinone (**85**), using formamidine acetate.

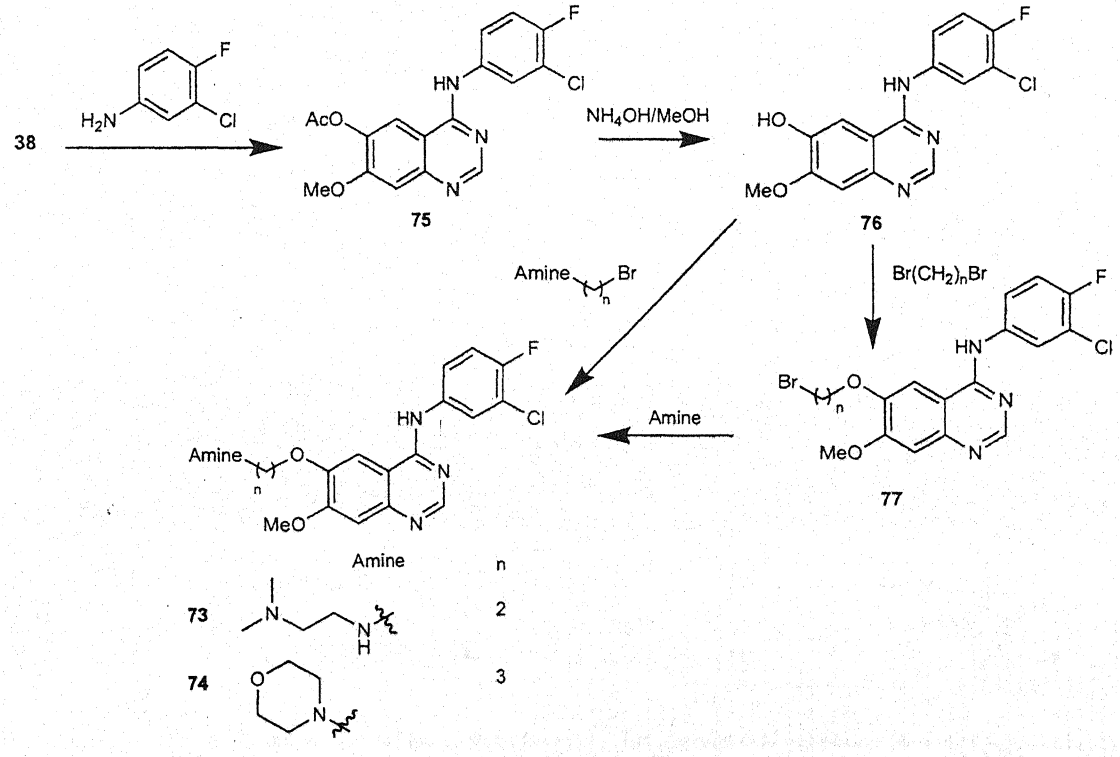
Compound **81** was obtained from **85** in two ways. Firstly, compound **85** was reacted with dimethylamine to provide dimethylaminopyridopyrimidinone (**86**), which was chlorinated and then reacted with 1-benzyl-1*H*-indazole (**88**) to provide compound **81**.

In the second approach, compound **85** was chlorinated and reacted with 1-benzyl-1*H*-indazole (**88**) to afford compound **87**, which upon reaction with dimethylamine provided compound **81**. 1-Benzyl-1*H*-indazole (**88**) was synthesized from 5-nitro-1*H*-indazole (**89**) as shown in scheme **15** [34]. Compound **89**, upon *N*-benzylation provided mixture of compounds **90** and **91**. 1-Benzyl-5-nitro-1*H*-indazole (**91**) was isolated and then hydrogenated by Pd/C to provide amino indazole **88**.

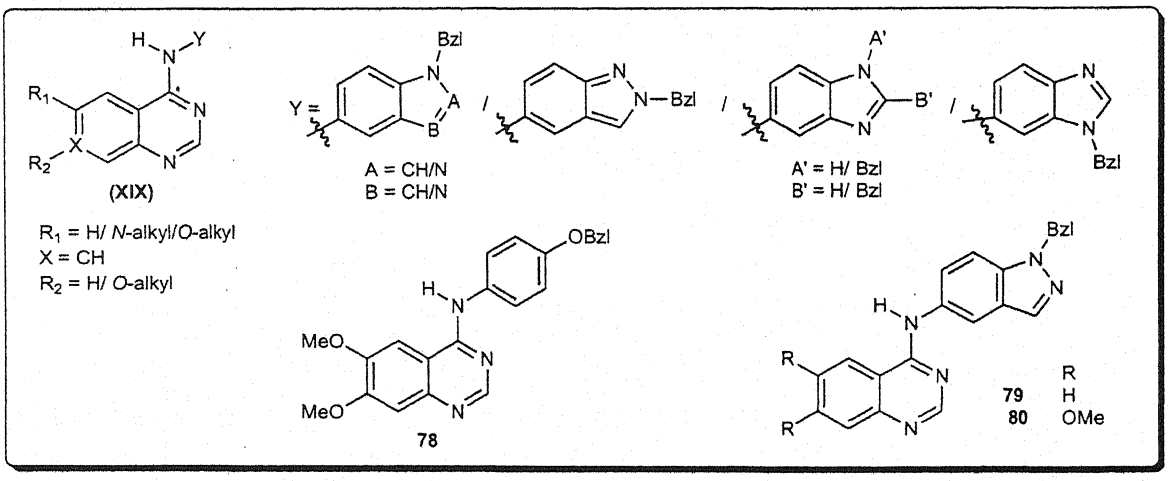
Albuschat *et al.* synthesized various anilinoquinazoline derivatives of prototype **XX** as **92**, **93** and **94** [35]. The study was based on the EGFR tyrosine kinase inhibitory activity of levendustin (**92**) and salicylanilides derivatives **93** and **94** [36].

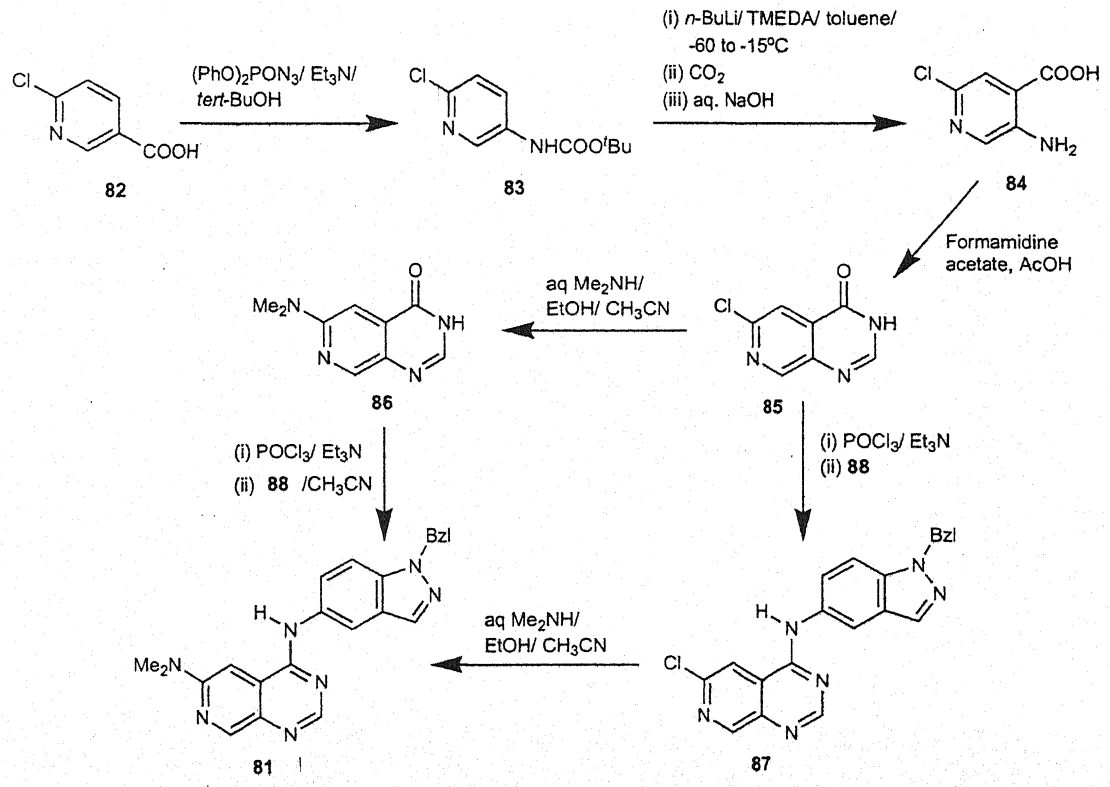
Based on the bioisosteric relation between salicylic and quinazoline group, quinazoline derivatives **95** and **96** were synthesized. These anilinoquinazoline derivatives were further tried to convert into irreversible EGFR inhibitors, which contain the Michael acceptor position similar to CI-1033 (**54**).

Compounds **95** and **96** showed observable inhibitory effects on U87MG, A172 T98G cell lines and potent EGFR tyrosine kinase inhibitory activity with IC_{50} in the range of 0.1-1 μM. Compound **96** exhibited high activity against CCRF-CEM (GI_{50} = 0.04 μM, TGI = 0.09 μM) and A498 (GI_{50} = 0.23 μM). The LC_{50} value on leukemia cancer cell line was observed 33.7-98.7 μM with com-

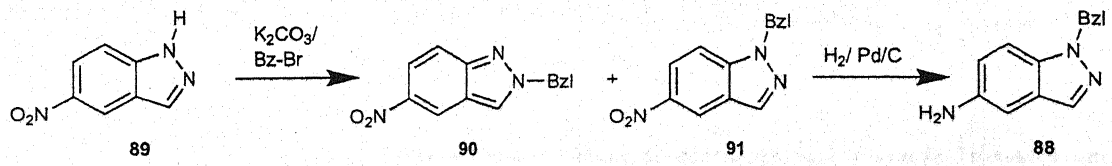


Scheme 13.





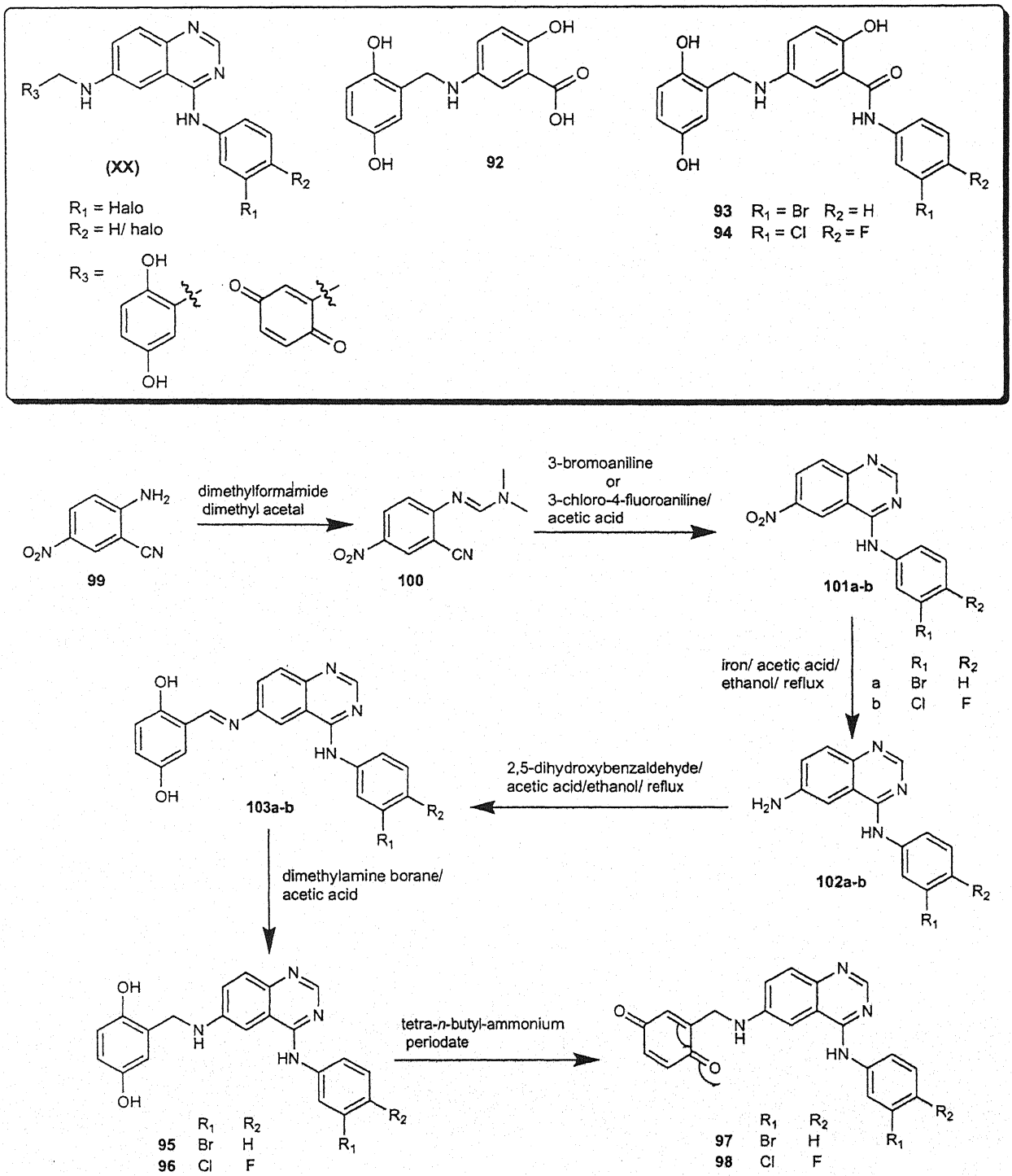
Scheme 14.



Scheme. 15.

pound **96**. Both the compounds **95** and **96** showed LC_{50} value of 51.5-98.7 μM in NSCLC, 61.4-74.1 μM in melanoma and 42.4 & 40.8 μM in renal A498 cancer cell lines, respectively.

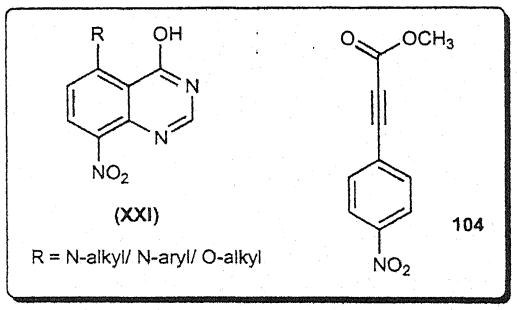
The preparation of compounds **95** and **96** from 5-nitroanthranilo nitrile (**99**) is shown in scheme **16** [35]. Compound **99** was reacted with dimethylformamide dimethylacetal to afford compound **100**,



Scheme 16.

which on treatment with the desired amine to furnish 6-nitro-4-phenylaminoquinazoline (**101a-b**). The nitro group of compound **101a-b** was reduced to 6-aminoquinazoline **102a-b** using iron in acetic acid. Compound **102a-b**, upon reaction of 2,5-dihydroxy benzaldehyde, provided Schiff base (**103a-b**). The imine group reduction of **103a-b** with dimethylamine borane in acetic acid furnished the desired compounds **95** and **96**. To obtain the irreversible EGFR tyrosine kinase inhibitor (compounds **97** and **98**), hydroquinone moiety of compounds **95** and **96** was oxidized with tetra-*N*-butylammonium periodate but the compounds **97** and **98** were not obtained in pure form due to instability occurred by rearrangement reaction.

Jin *et al.* synthesized a number of 5–sustituted-4-hydroxy-8-nitroquinazoline derivatives of prototype **XXI** that caused the inhibition of both EGFR and ErbB-2 tyrosine kinases [7].



The study was based on molecule B-17 (**104**), an inhibitor of ErbB-2 TK [37]. The nitro group has been substituted at C-8 position of the 4-hydroxyquinazoline (**105** and **106**). Structure activity relationship studies indicated that aniline moiety is essential at C-5 position. Further substitution at C-4' position in aniline ring with electron donating group exhibited a positive remarkable effect for example, 4'-methoxy substituted compound (**105**) showed best dual inhibition of both EGFR and ErbB-2 signaling with $IC_{50} < 0.01$ and 13.0 μM, while large benzyloxy substituted compound (**106**) caused more inhibition of ErbB-2 signaling with IC_{50} of 7.0 μM. Compounds **105** and **106** showed promising antiproliferative effect against the EGFR and ErbB-2-overexpressing tumor cell lines.

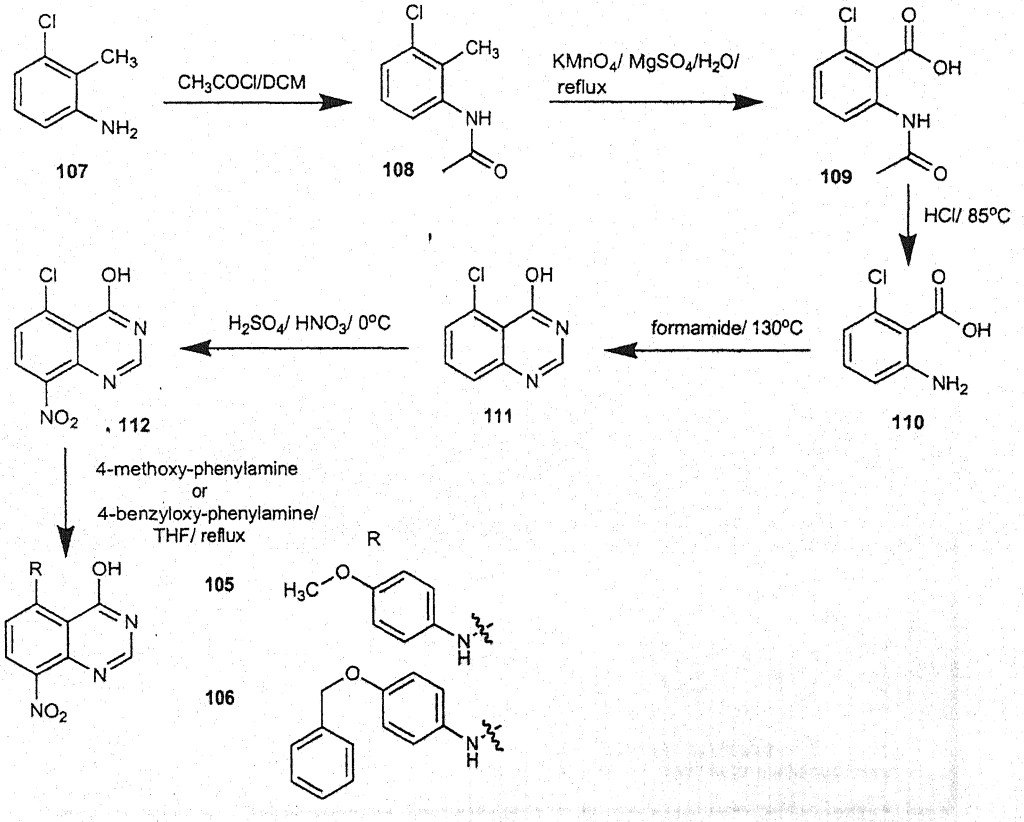
Compounds **105** and **106** were synthesized from 3-chloro-2-methyl aniline (**107**) as shown in scheme **17** [7]. Acetylation of amino group in **107** with acetyl chloride provided *N*-(3-chloro-2-methyl-phenyl)-acetamide (**108**), which was oxidized to 2-Acetylamino-6-chloro-benzoic acid (**109**) using $KMnO_4$. The deacetylation of **109** was carried out by HCl to afford anthranilic acid **110**, which on cyclization with formamide provided 5-chloroquinazoline (**111**). Nitration of **111** with nitric acid afforded 8-nitroquinazoline (**112**), which on reaction separately with 4-methoxy-phenylamine and 4-benzyloxy-phenylamine furnished compounds **105** and **106**, respectively.

Ballard *et al.* synthesized various 5-substituted 4-aminoquinoline derivatives of prototype **XXII** as ErbB2 receptor tyrosine kinase inhibitor [38]. The study was based on the lead compound **113**, which has IC_{50} of 0.0056 μM for inhibition of ErbB2 kinase enzyme [39]. Aniline side chain of compound **113** was assumed to be interacting with the selective pocket at erbB2 active site. Based on the homology model and work carried out on inhibitors of c-Src, substituent was shifted from C-6 position to C-5 in quinazoline, which can now occupy kinase sugar pocket and improve the affinity towards ErbB2.

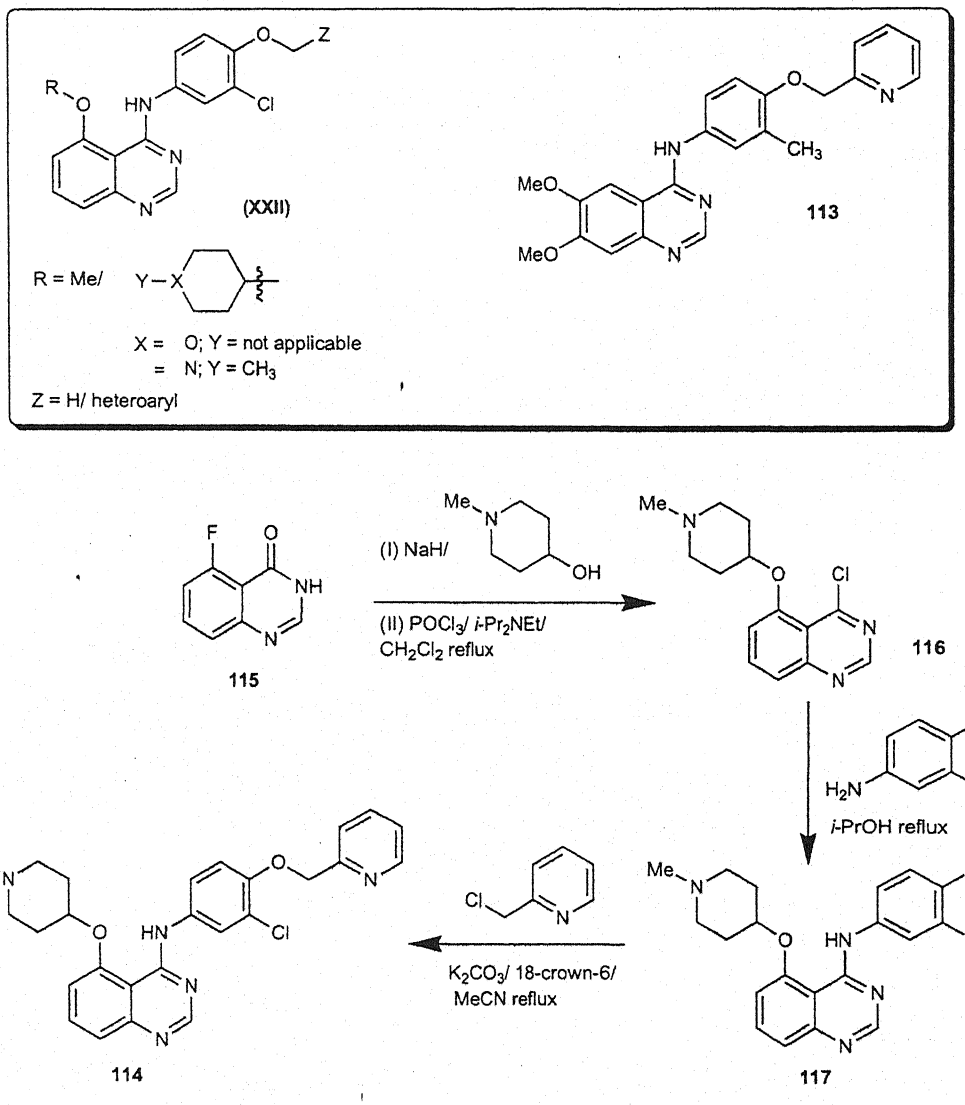
Structure activity relationship studies presented that the transferring of substituent from C-6 position to C-5, introduction of a cyclic amine and changing the *meta*-substituent in the extended aniline from methyl to chlorine, significantly increased the affinity for both erbB2 and EGFR kinases. Further, upon removal of C-7 methoxy group provided the selectivity for erbB2.

Compound **114**, however identified as potent compound, which showed IC_{50} <0.002, 0.14, 0.027 and 0.46 μM on erbB2, EGFR, BT474C and KB cell line, respectively. The synthesis of compound **114** from starting material 5-fluoro-4-(3*H*)-quinzolone (**115**) is depicted in scheme **18** [38]. *O*-alkylation of **115** at C-5 was followed by chlorination afforded compound **116**.

The C-4 chloro group in compound **116** was replaced by 4-amino-2-chlorophenol to provide compound **117**, which on treat-



Scheme 17.

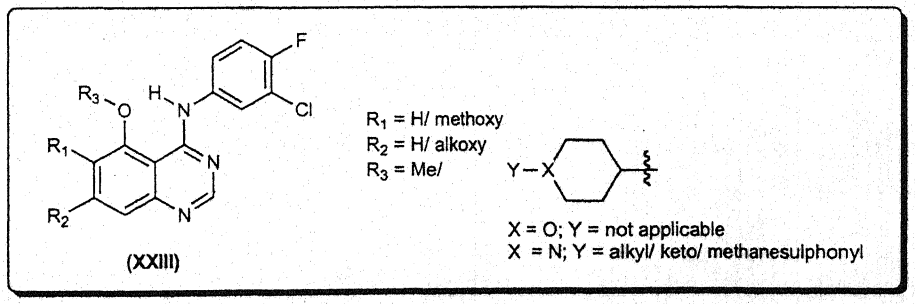


Scheme 18.

ment with 2-chloromethylpyridine in base assisted medium furnished compound **114**.

Ballard *et al.* realized that little work is done at C-5 in anilinoquinazolines as EGFR inhibitors and synthesized various molecules of prototype **XXIII** [39].

Simple substitutions at C-5 position in quinazoline decreased the activity and generally, this position is kept unsubstituted. On examining the overlay of ATP with the known binding mode of anilinoquinazoline, it was predicted that potent inhibitor could be obtained on placing the appropriate substituent at C-5 position, which can utilize the ribose-binding pocket.



Structure activity relationship studies showed that at C-5 position, basic cyclic group exhibited potent enzyme inhibition, which may be due to electrostatic complementaries with the cationic side chain. Cyclic group, without this charge, were lacking in the activity. Similarly, open chain analogue also exhibited less activity. Moving the methoxy from C-7 to C-6 position decreased the activity. Compound **118** showed IC_{50} of 21 nM on EGFR and 87 nM on EGF-driven KB cell line.

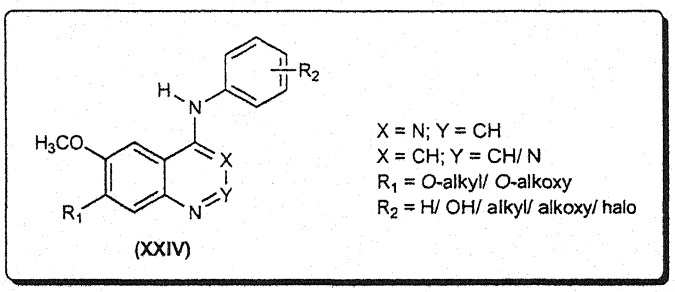
Compound **118** was synthesized from **119** as shown in scheme **19** [40]. Anthranilic ester **119** was cyclized to quinazolone (**120**) by formamide acetate, which was selectively demethylated (**121**) at C-5 position by magnesium bromide in pyridine. Compound **121** was protected with pivalate group to provide compound **122**, which upon Mitsunobu reaction with 4-hydroxy-1-methylpiperidine afforded C-5 substituted quinazolone followed by the removal of pivalate group with liquid ammonia to afford **123**. The chlorinatation of **123** with phosphorus oxychloride gave 4-chloro derivative, which was allowed to react with 3-chloro-4-fluoro aniline to afford the target molecule (**118**).

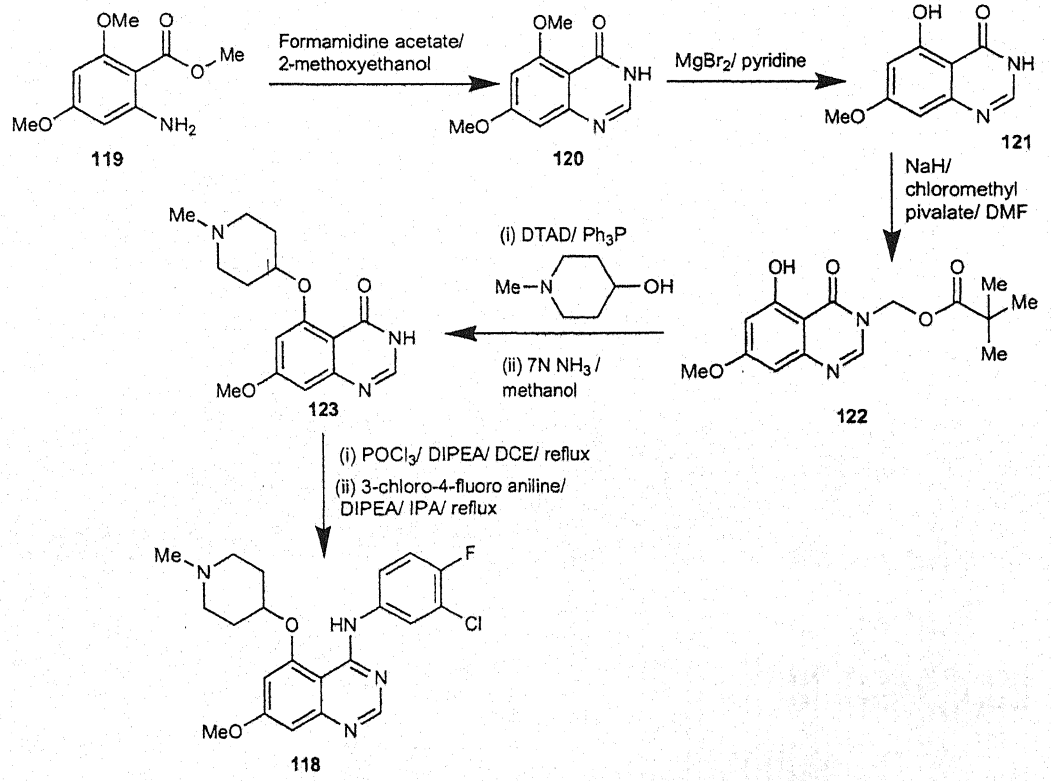
2.2. VEGFR Inhibitors

Hennequin *et al.* has developed substituted 4-anilinoquinazolines and related compounds of prototype **XXIV** as VEGFR inhibitors [41].

Structure activity relationship studies showed that small lipophilic substituents are preferred at C-4' position like halogen or methyl, whereas the small substituents like hydrogen or fluorine are preferred at C-2' position. Introduction of the hydroxy group in aniline produced the highly potent compounds (e.g. **124-127**). Further, modifications at C-7 position also provided potent compounds.

Two endothelium associated, high affinity RTK's for VEGF have been identified, the fms-like tyrosine kinase receptor, Flt, and the kinase insert domain-containing receptor, KDR (also referred to as Flk-1 in mice). Compound **124** and **125** showed IC_{50} value of 0.003 μM on Flt cell line. Compound **126** exhibited IC_{50} value of < 0.002 μM on Flt and KDR cell line and 0.004 μM on VEGFR cell line. Compound **127** inhibited the growth of Calu-6 lung carcinoma xenograft upto 75%.

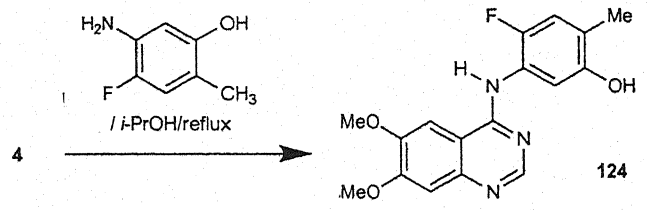




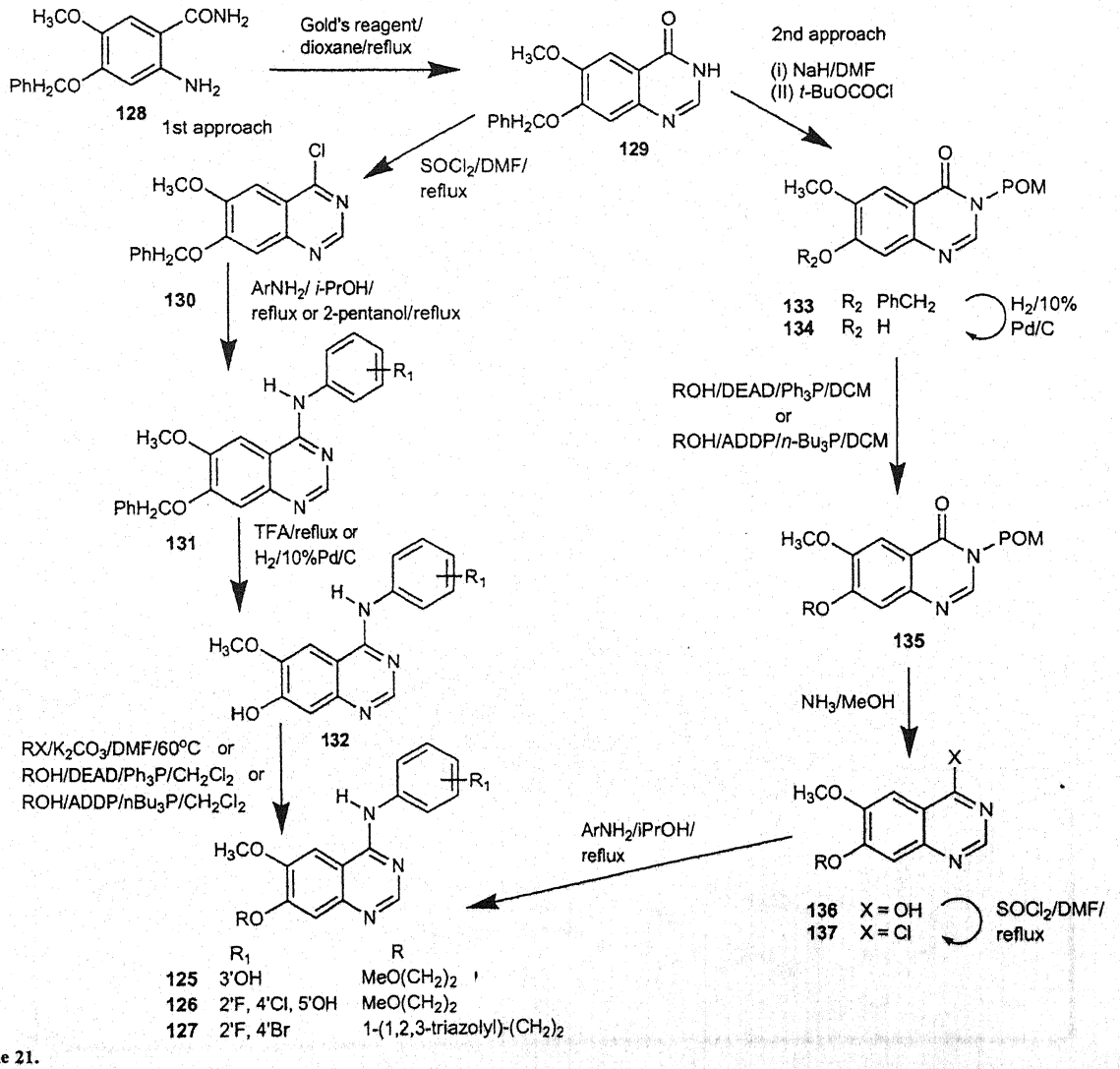
Scheme 19.

Compound **124** was synthesized from 6,7 dimethoxy-4-chloroquinazoline (**4**) as the starting material as shown in scheme **20** [41]. Compound **4** upon reflux with 5-amino-4-fluoro-2-methylphenol afforded compound **124**. Compounds **125**, **126** and **127** were prepared from aminobenzamide derivative (**128**) [42] as shown in scheme **21** [41]. Compound **128** was converted to 7-benzyloxy-3,4-dihydroquinazolin-4-one (**129**) by Gold's reagent in dioxane.

Two routes starting from compound **129** were employed to achieve synthesis of **125**, **126**, and **127**. In the first route, compound **129** was chlorinated at C-4 position (compound **130**) and reacted with the particular aniline (**131**). Benzyl group at C-7 position was removed by TFA or by hydrogenation to afford compound **132**. The suitable side chain at C-7 position was introduced either by the direct alkylation or by Mitsunobu reaction to afford compound **125**, **126**, and **127**. The second approach was also employed, in which C-7 side chain was attached prior to aniline ring. The quinazolinone **129** was protected at N-3 position using POM (pivaloyloxymethyl) group (compound **133**). Benzyl group was removed from C-7 position (compound **134**) and side chain was introduced at C-7 position (compound **135**). POM group was removed under basic conditions (compound **136**) and chlorination was carried out at C-4 position (compound **137**). Chloro group was further displaced with desired amine to afford compounds **125**, **126**, and **127**.



Scheme 20.



Scheme 21.

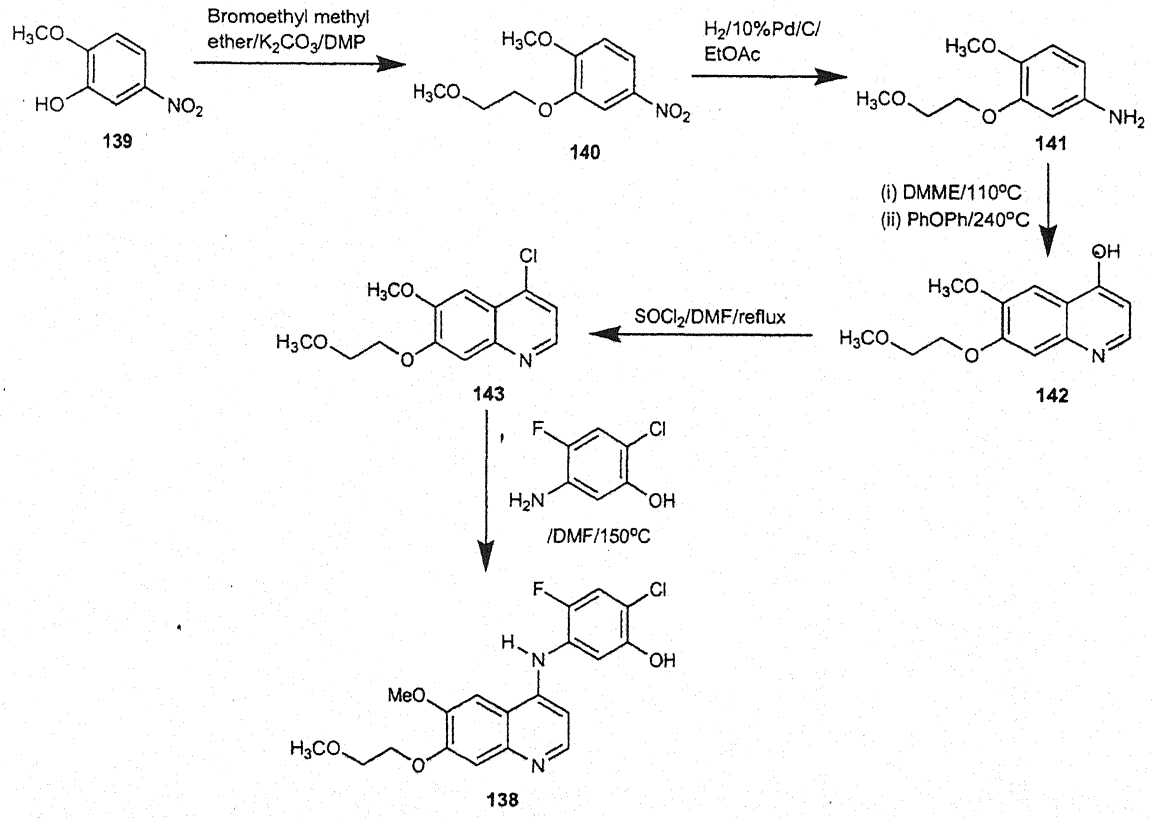
4-Anilinoquinoline derivative (**138**) was prepared from 2-methoxy-5-nitrophenol (**139**) as shown in scheme **22**. The alkylation of **138** with 1-bromo-2-methoxyethane afforded compound **140**, which was reduced to amino derivative **141** with H_2 in Pd/C. Compound **141** was cyclized to quinoline intermediate (**142**) with diethylethoxymethylene malonate under reflux condition. Compound **142** was chlorinated at C-4 position to furnish compound **143**, which was reacted with 5-amino-2-chloro-4-fluoro-phenol to afford compound **138**.

Compound **138** showed IC_{50} value of 0.003 and < 0.002 μM on Flt and KDR cell line.

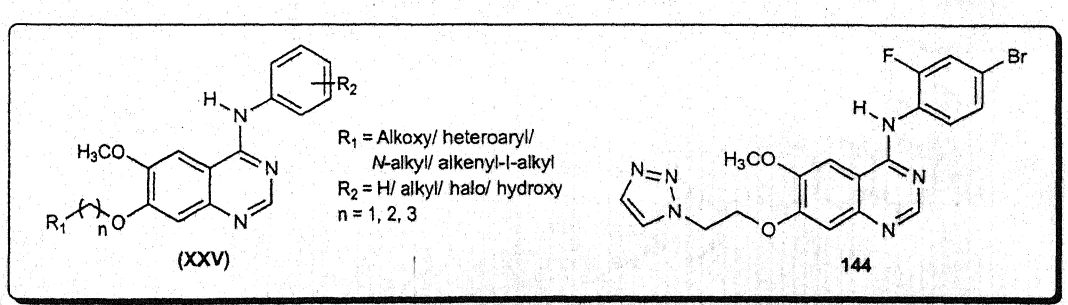
Hennequin *et al.* synthesized various quinazoline derivatives of prototype **XXV** [43], based on the earlier observations that aniline ring consisting of small lipophilic electron deficient substituent at the C-2' position along with larger lipophilic electron withdrawing atom at C-4' position showed potent activity.

Structure activity relationship studies exhibited that the replacement of the C-4' hydrophilic electron withdrawing substituent by more lipophilic group have increased the C-π character and leads to improvement in both Flt-1 and KDR inhibition. Introduction of fluorine atom at C-6' position increased the selectivity for the Flt-1 over KDR. This pattern earlier leads to the active compound **144** (ZD4190) in which C-7 chain was neutral [41]. Replacement of this neutral side chain by the basic *N*-methylpiperidine (compound **145**) leads to excellent level of KDR inhibition with improved physicochemical properties and plasma half-life. Compound **145** showed IC_{50} of 0.04 μM and 1.6 μM on KDR and Flt-1 cell lines, respectively.

The synthesis of compound **145** from the *N*-3-POM protected-7-hydroxy-quinazoline (**134**) is depicted in scheme **23** [43]. Compound **134** was reacted with N-protected 4-piperidine methanol to afford compound **146**, the removal of Boc-group of which with TFA resulted **147**. *N*-methylation of **147** with formaldehyde under



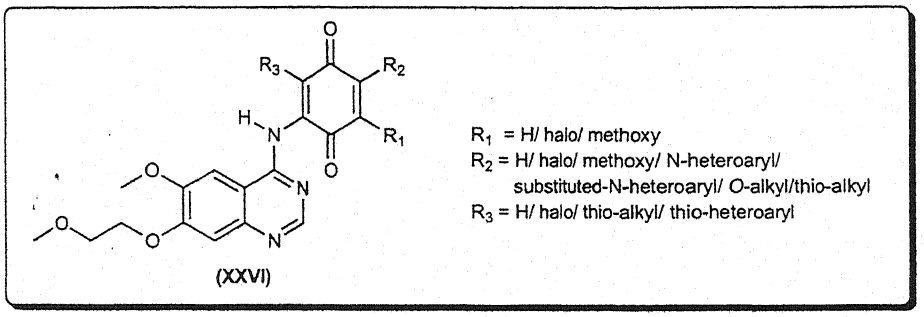
Scheme 22.

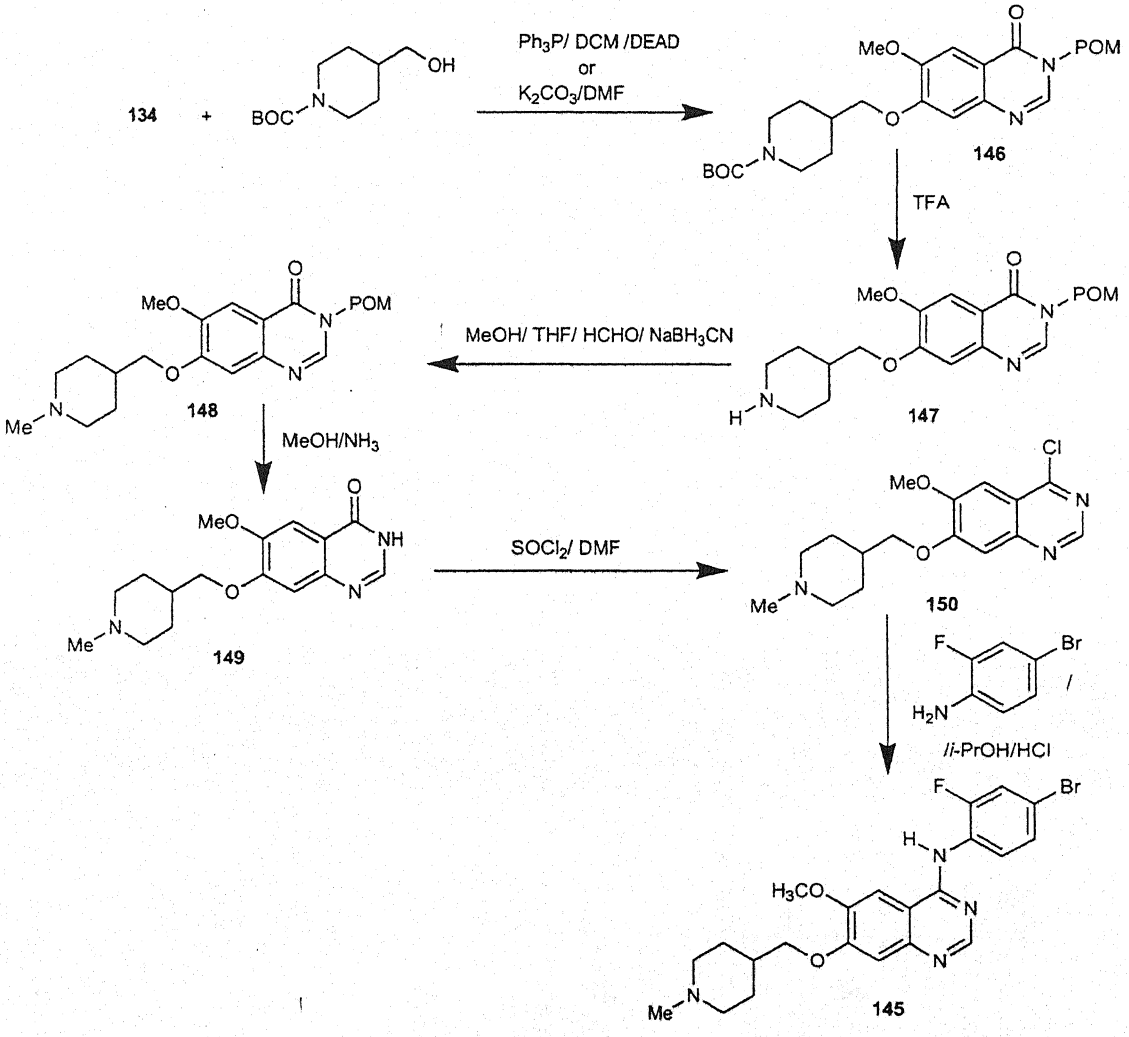


reducing conditions provided compound **148**. The POM-group was removed by ammonia in methanol to furnish quinazoline **149**. Compound **149** was chlorinated with thionyl chloride to yield compound **150**, which was allowed to react with 4-bromo-2-fluorophenyl aniline to give the desired molecule (**145**).

Wissner *et al.* synthesized quinazolines of prototype **XXVI** with different reactive groups attached at C-4-position and studied the covalent binding inhibition of VEGFR-2 [8].

Compound **151** showed IC_{50} of 5.1 nM in presence of 10 μM concentration of ATP. In presence of 100 μM of glutathione and 10 μM concentration of ATP, IC_{50} of compound **151** was found to be 6.9 nM. Compound **151** was also tested in other kinases like AKT, BTK, CDK4, EGFR, GSK, IGFR, IKK, ITK etc and in these assays, IC_{50} varied between 1.8 to >117 μM. These activity results prompted its *in vivo* evaluation.





Scheme 23.

The oral administration of **151** resulted significant reduction in the tumor growth. Efficacy of the **151** is comparable to Bevacizumab, which is a neutralizing antibody to VEGF.

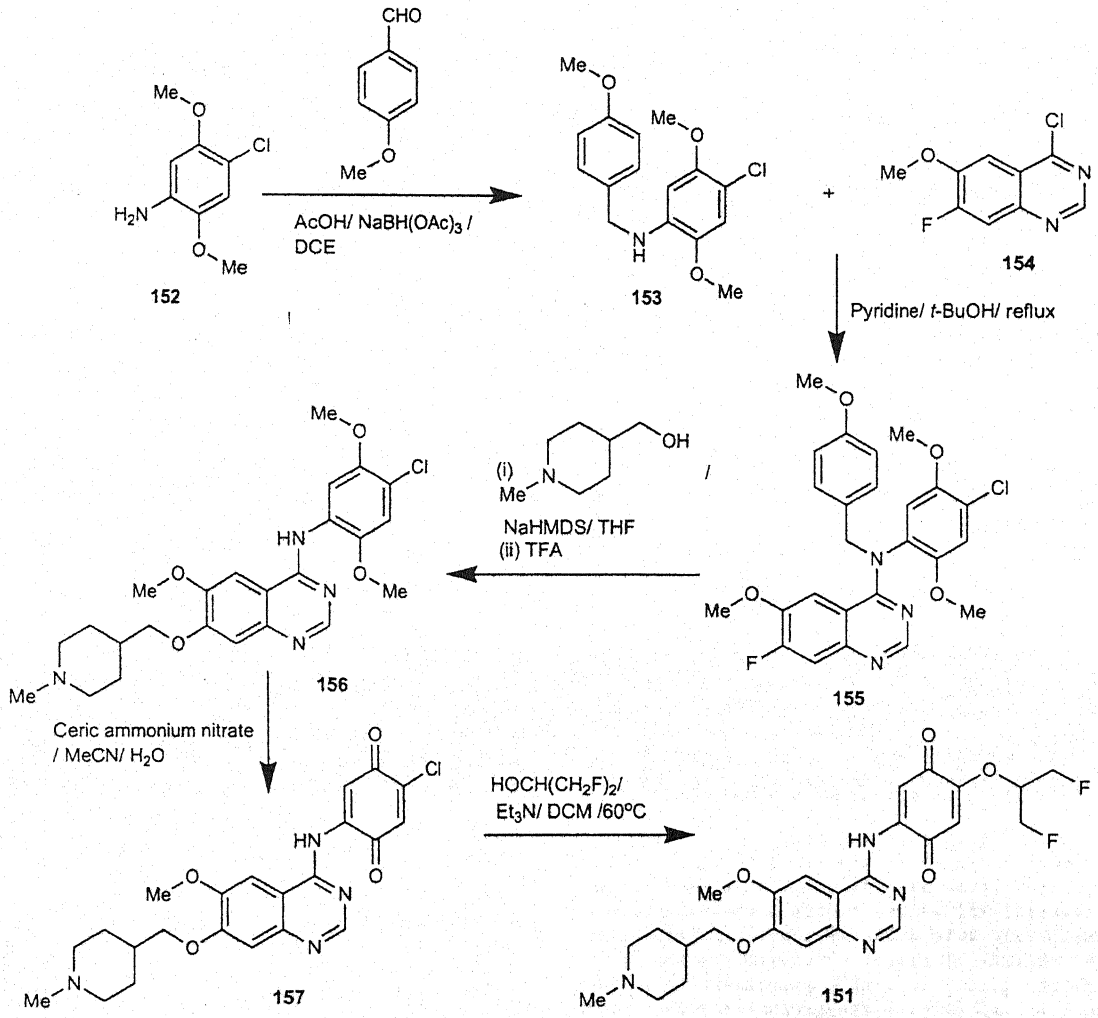
The preparation of compound **151** from 4-chloro-2,5-dimethoxy phenylamine (**152**) is shown in scheme **24** [8]. The reaction of **152** with 4-methoxy benzaldehyde afforded compound **153**, which on condensation with 4-chloro-7-fluoro-6-methoxyquinazoline (**154**) [44] in pyridine furnished compound **155**. (1-Methyl-piperidin-4-yl)-methanol was reacted with fluorine atom of **155** and the resulting ether compound was treated with TFA to provide compound **156**. Compound **156** was oxidized with ceric ammonium nitrate to yield quinone **157**. The chloro group of **157** was replaced with 1,3-difluoro-propan-2-ol to give the desired compound **151**.

2.3. PDGFR Inhibitors

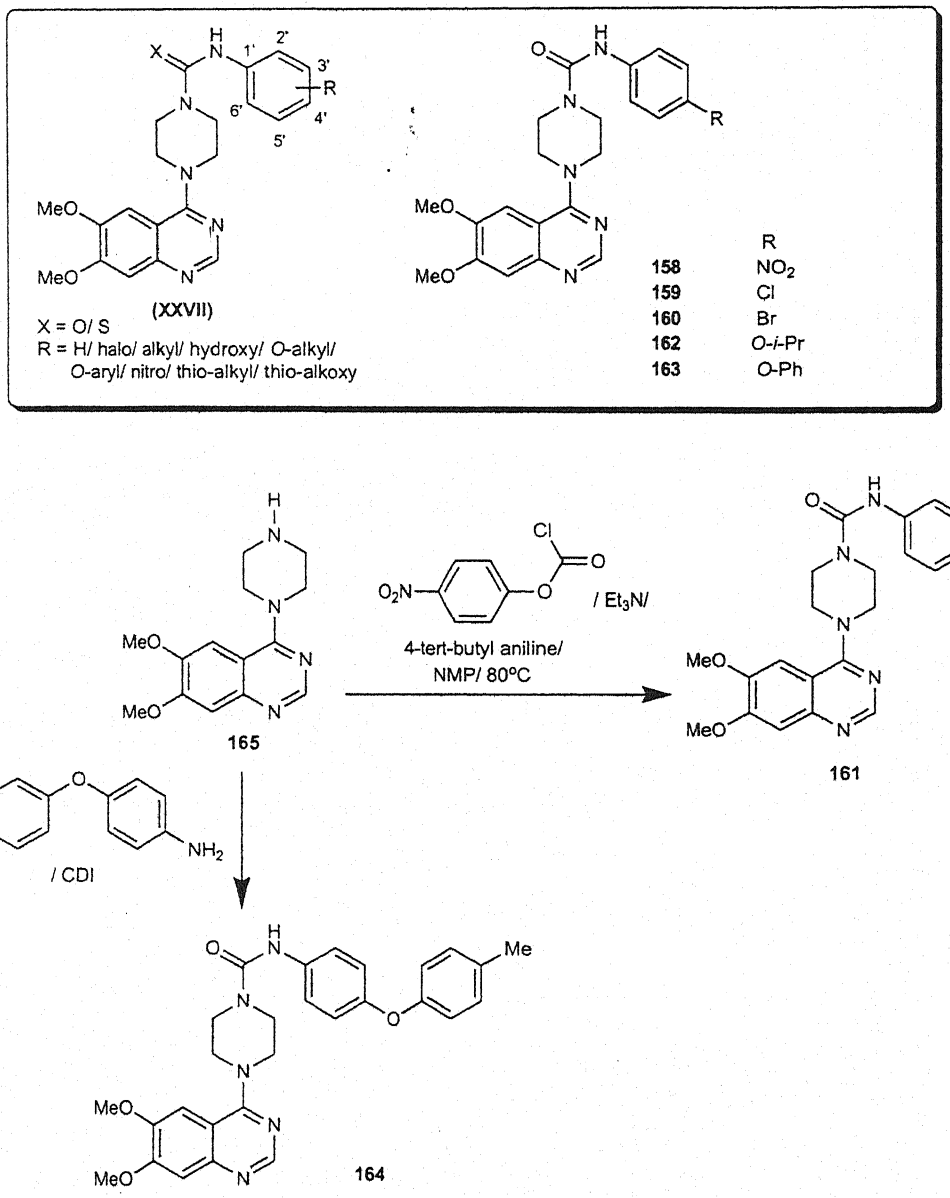
Matsuno *et al.* carried out synthesis of various quinazolines derivatives of prototype **XXVII** [45]. This study was based on the potent anticancer molecule KN1022 (**158**) [46], in which the modifications were carried at 4-nitrophenylurea moiety. These molecules were evaluated for the inhibition of β-PDGFR phosphorylation. Structure activity relationship studies in the series exhibited that position and nature of the substituents on the phenyl ring attached to urea moiety have a substantial influence on the inhibitory activity.

Substitution at C-4' in phenyl ring was the most favorable, while the activity was reduced upon incorporating the substitutions at C-3' and C-2' positions. Bulky hydrophobic substituents at C-4' position increased the activity. Small substituent like methyl at bulky phenoxy group (compound **164**) is also exhibited potent activity. The introduction of hydrophilic substituent at C-4' is unfavorable. Thiourea derivative also showed inhibitory activity on β-PDGFR phosphorylation but lesser than ureas. However, compounds **159-164** exhibited IC_{50} of 1.10, 0.53, 0.03, 0.23, 0.08 and 0.02 μmol/L in comparison to 0.70 μmol/L of **158** for the inhibition of β-PDGFR phosphorylation. Compounds **159**, **160** and **163** were also evaluated for the inhibition of SMC proliferation induced by PDGF-BB. Compounds **159**, **160** and **163** showed good oral absorption and high plasma drug concentration and significant inhibition of neointima formation in the range of 24-38%, which can be useful for the treatment of the atherosclerosis.

The synthesis of most active compound **161** is described in scheme **25** [45]. A mixture of **165** [47], 4-*tert*-butylamine, NMP and 4-nitrophenyl chloroformate in presence of triethylamine was refluxed to provide **161**. The reaction of 4-*p*-tolyloxy phenylamine



Scheme 24.



Scheme 25.

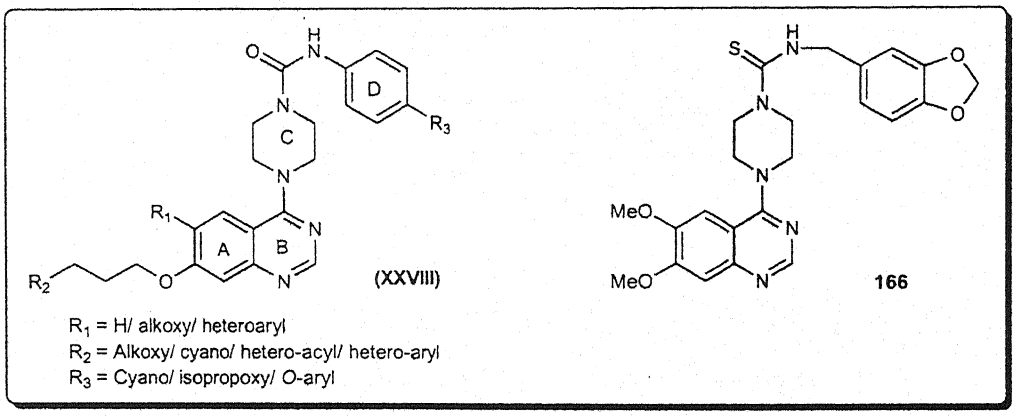
with **165** in presence of *N,N'*-carbonyldiimidazole (CDI) furnished **164**.

Pandey *et al.* synthesized various β-PDGFR phosphorylation inhibitors of prototype **XXVIII** [48]. The study was based on the existing 4-piperazinylquinazoline derivative CT52923 (compound **166**) [49, 50]. The aim was to increase the potency, appropriate kinase specificity including high oral bioavailability and long plasma half-life.

Various modification were carried out at A and D ring. In the D ring, 4-isopropoxy or cyano substituent provided the compounds with maximum metabolic stability, oral bioavailibility and plasma half-life. In the A ring, the presence of propoxy basic side chain at C-7 position leads to improved aqueous stability, bioavailability, improved inhibitory activity, reduced protein binding and good potency. Simultaneously, upon replacement of urea linkage by isosteric thiourea unit showed good inhibitor activity.

Compound **167** showed IC_{50} value of 26 and 36 nM on MG63 cell line in the absence and presence of the plasma, while IC_{50} value is 200 nM in Chinese hamster ovary cell line. Compound **167** also inhibits autophosphorylation of a constitutively activated Flt3/ITD mutant expressed in hematopoietic cells or AML cell lines with IC_{50} value of 30-100 nM.

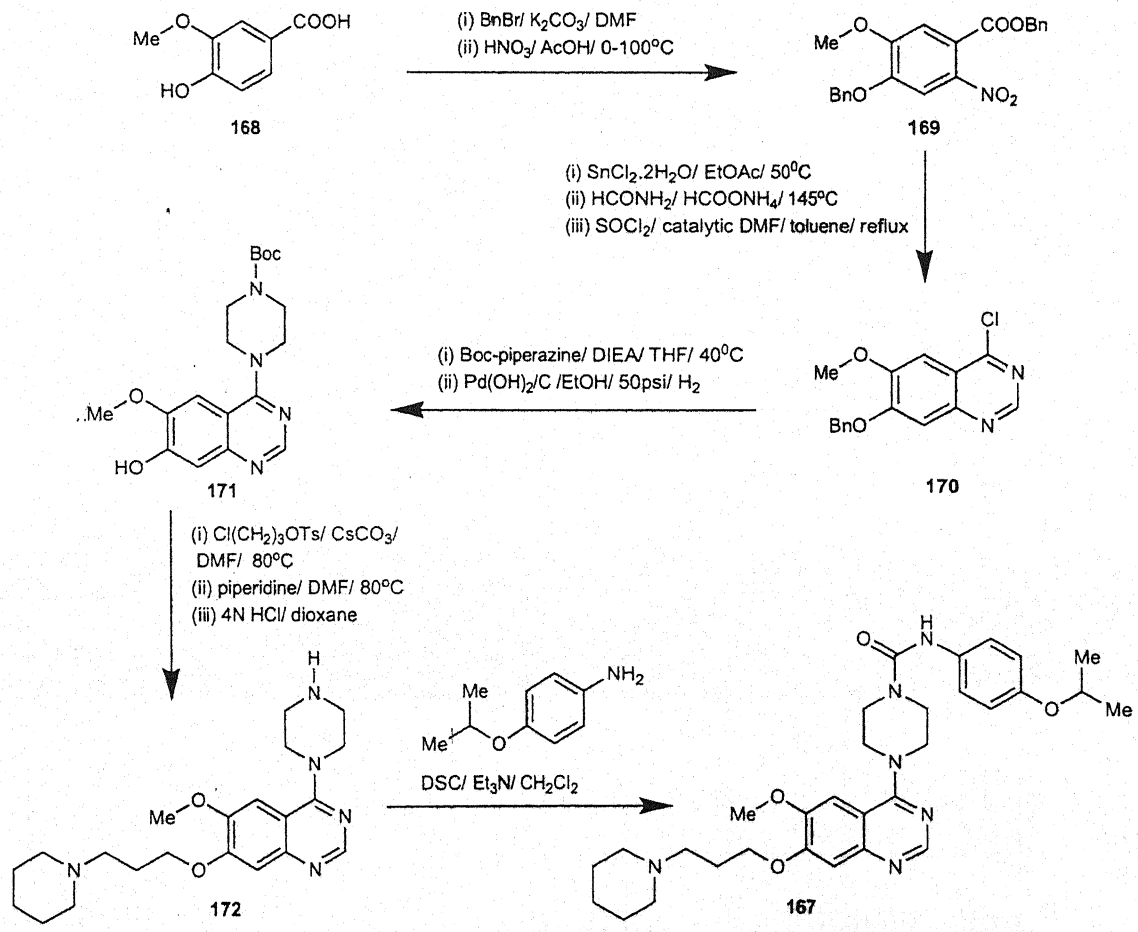
Compound **167**, was synthesized starting from vanillic acid (**168**) as shown in scheme **26** [47]. Benzyl ester of vanillic acid was prepared from the reaction of **168** with benzyl bromide and then nitrated by nitric acid to afford compound **169**. The nitro ester **169** was reduced with stannous chloride to amino intermediate, which was cyclized with formamide to provide the quinazoline derivative followed by the chlorination of the obtained compound with thionyl chloride to afford 4-chloroquinazoline **170**. The C-4 chloro group was replaced by the Boc-piperazine and then debenzylated by hydrogenation to afford compound **171**. *O*-alkylation of **171** was car-



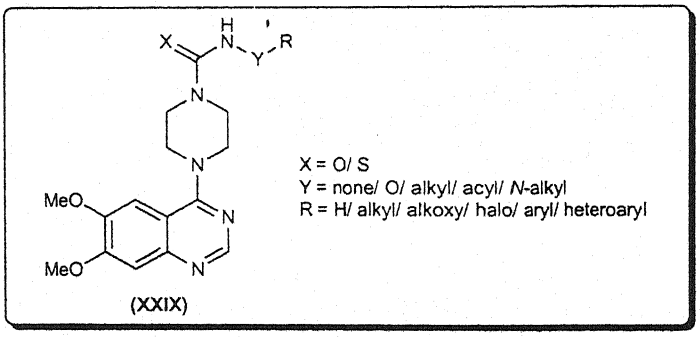
ried out at C-7 position and the Boc-group was removed by hydrochloric acid to give compound **172**. Urea analog (**167**) of compound **172** was prepared by its reaction with 4-isopropoxy-phenylamine and DSC (*N,N'*-disuccinimidyl carbonate) followed by amine.

Matsuno *et al.* carried out the synthesis of similar quinazoline derivatives of prototype **XXIX** and studied Structure activity relationship for inhibition of β-PDGFR phosphorylation [51]. The designing of the molecules was based on the molecule KN1022 (**158**), which is a potent inhibitor PDGFR phosphorylation. Structure activity relationship studies indicated that the change of the linker between the phenyl ring and the (thio)urea moiety has a major effect on the potency. In the urea derivatives, insertion and extension of the methylene chain reduced the activity and which is opposite in case of benzylthiourea derivatives.

Benzylthiourea with relatively small substituents on C-4' such as compounds **173-175** and 3,4-methylenedioxy group such as



Scheme 26.



(XXIX)

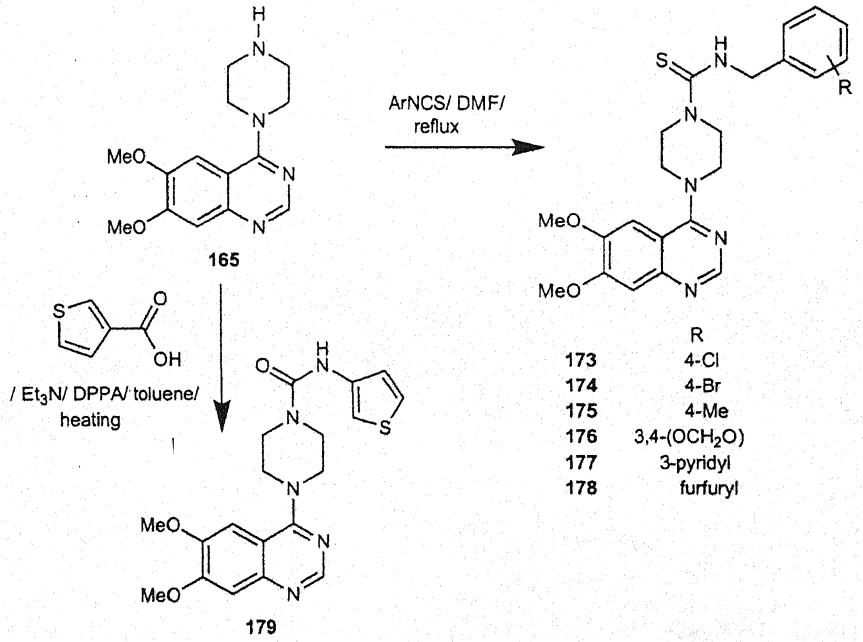
compound KN734 (**176**) were found to be most suitable. Upon replacement of the phenyl ring with other heterocyclic ring system, improvement in the aqueous solubility has been observed. Thienyl analogue (**179**) showed activity similar to **158**. Compound **177** possessing 3-pyridine ring with methylene bridge and furfuryl ring derivative (**178**) showed high aqueous solubility. It was observed that acidic hydrogen on the (thio)urea moiety is essential for interaction with PDGFR. Modification in the piperazine ring had no positive influence on the activity. Compounds **173**, **174**, **175** and **176** exhibited IC_{50} value of 0.07, 0.03, 0.03 and 0.09 μmol/L, respectively, on β-PDGFR inhibition. Compounds **176**, **178** and **179**, however, showed good oral absorption and high plasma concentration. These compounds also cause inhibition of neointima formation. These compounds may represent a new approach for atherosclerosis treatment.

The synthesis of compounds **173-178** was achieved from 4-(1-piperazinyl)-6,7-dimethoxy-quinazoline **165** [44] as shown in scheme **27** [51]. Compound **165** was refluxed with appropriate substituted isothiocyanate in DMF to provide compounds **173-178**. Compound **179** was synthesized by the reaction of compound **165** with 3-thiophenecarboxylic acid in presence of DPPA and triethylamine as a base.

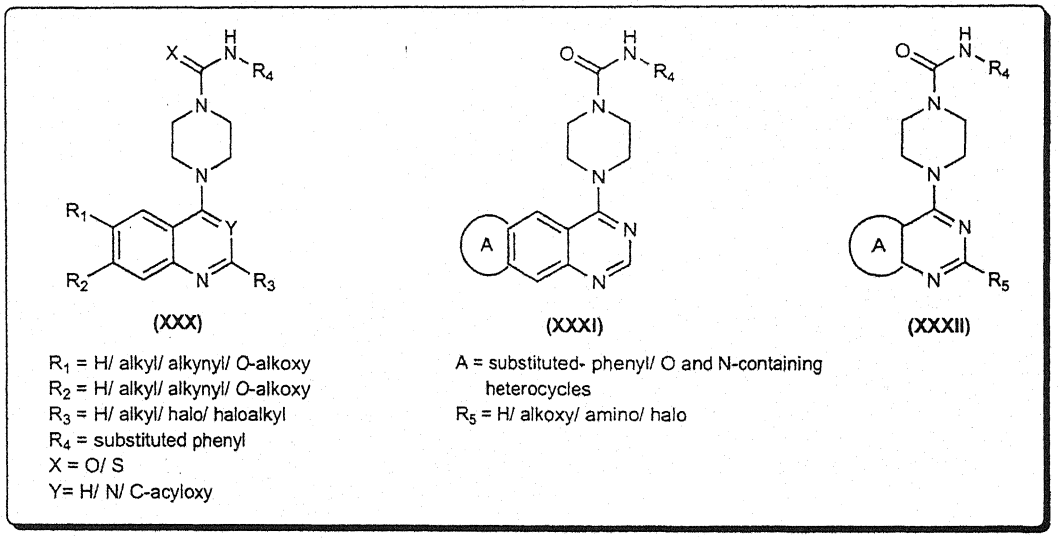
Based on the previously known PDGFR phosphorylation inhibitor, active molecules KN1022 (**158**) and KN734 (**176**) of quinazoline derivative, Structure activity relationship studies was carried out by Matsuno *et al.* and various compounds of prototype **XXX-XXXII** were designed and synthesized [52].

Structure activity relationship studies of the series resulted that the amongst C-6 and C-7-dialkoxy substituents, ethoxy analogue showed potent activity, which includes the compounds **180-184**. Amongst tricyclic quinazoline 2-oxoimidazo[4,5-*g*]quinazoline analogue, compound **185** exhibited potent activity. The exchange of the quinazoline ring with other heterocyclic ring furnished compounds pyrazolo[3,4-*d*]pyrimidine (**186**) and quinoline derivatives (**187**), which exhibited potent activity. However, isoquinoline pyridopyrimidine derivatives are found inactive. Therefore, it was concluded that the N-1 atom has an important role and replacement by N-7 and N-8 atoms in the parent quinazoline ring has detrimental effects. Metabolic polymorphism was also observed for the 6,7-dimethoxy substituents on the quinazoline ring. The introduction of 3-COOEt group to compound **187** had provided **188**, in which the activity was retained.

Compounds **180-184** showed the IC_{50} of 0.04, 0.02, 0.01, 0.01 and 0.02 μM against inhibition of β-PDGFR phosphorylation. The



Scheme 27.



tricyclic compound **185** showed IC_{50} of 0.10 μM and pyrazolo[3,4-*d*]pyrimidine derivative **186** exhibited IC_{50} of 0.17 μM. The 6,6-bicyclic-heterocycles **187** and **188** also exhibited IC_{50} of 0.18 μM and 0.09 μM, respectively for inhibition of β-PDGFR phosphorylation.

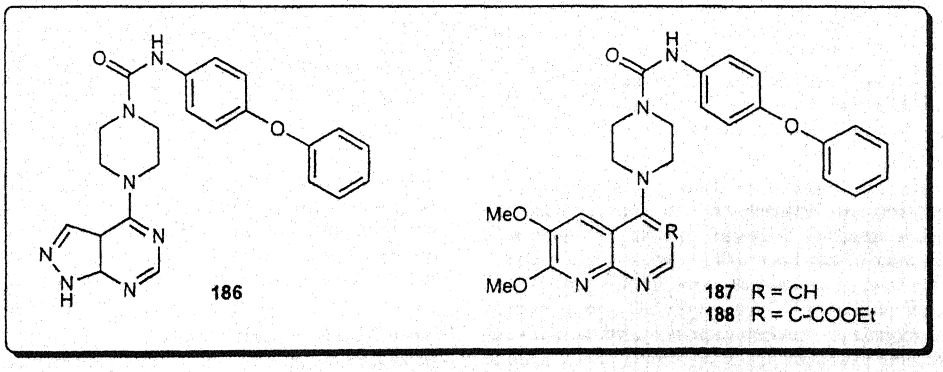
The C-6 alkoxy/C-7 methoxyquinazolines derivatives such as compounds **180/ 181** and C-6 methoxy/C-7 alkoxy quinazolines such as compounds **182-184** were synthesized from the isovanillic acid (**189a**) and vanillic acid (**189b**), respectively as described in scheme **28** [52]. On benzylation of compounds **189a-b**, with benzyl bromide provided corresponding benzyl derivatives. These benzyl derivatives on regioselective nitration with nitric acid followed by its reduction with zinc/ acetic acid afforded respective esters **190a-b**. Compounds **190a-b** upon cyclization with formamide furnished compounds **191a-b**. Chlorination of compounds **191a-b** with $POCl_3$ gave compounds **192a-b**, which on condensation with *N*-Boc piperazine afforded 4-(*N*-Boc-1-pierazinyl) quinazolines compounds **193a-b**. Deprotection of benzyl group in **193a-b** by hydrogenation provided compounds **194a-b**. The *O*-alkylation of **194a-b** with appropriate alkyl halide gave **195a-d**. The Boc-group in **195a-d** was removed by TFA in DCM and the resulting compounds on reaction with respective isocyanate furnished corresponding thiourea derivatives **180-184**.

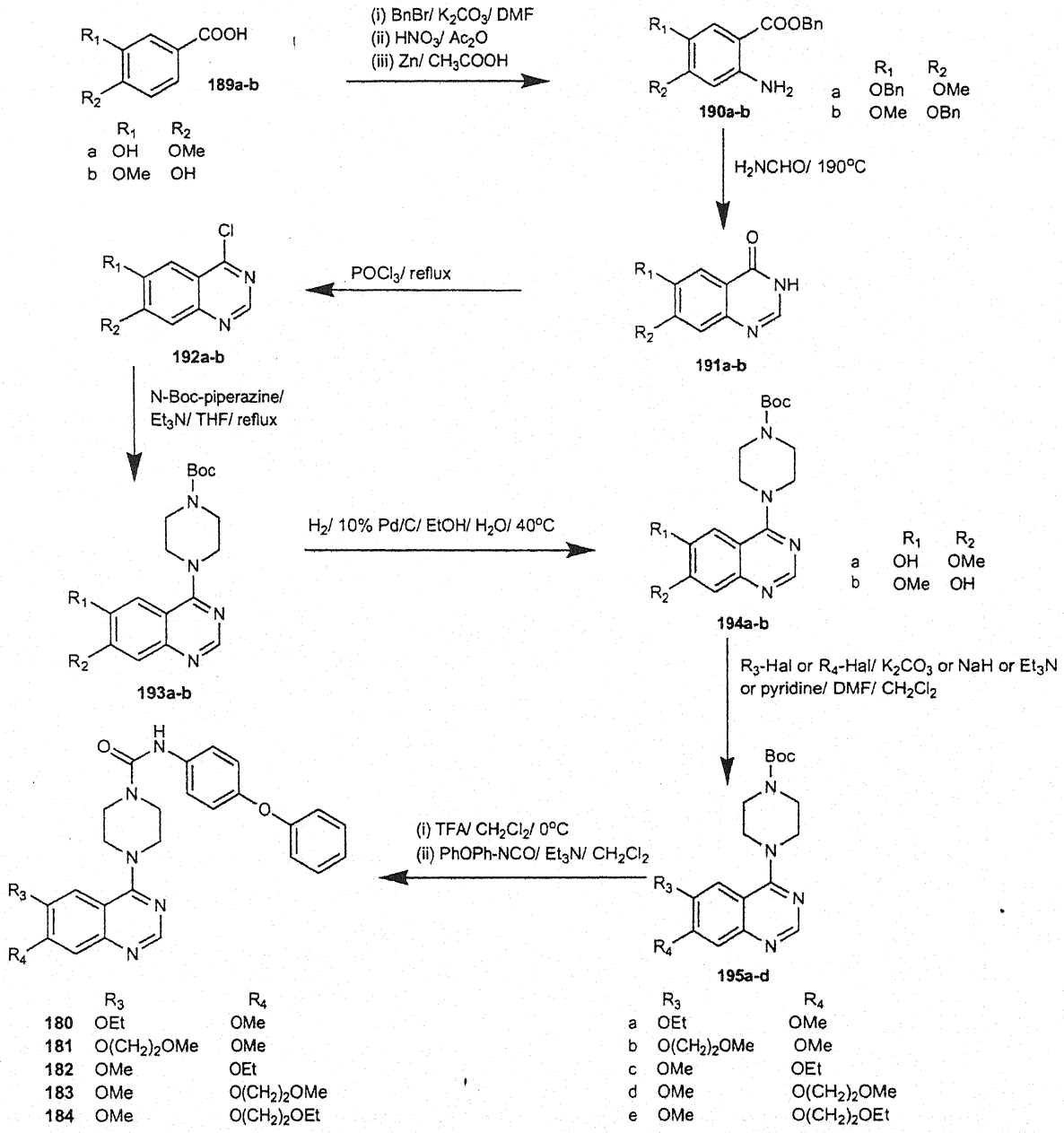
Compound **185** is synthesized from 3,4-diaminobenzoic acid methyl ester (**196**) as shown in scheme **29**. The compound **196** was treated with *N,N'*-carbonyldiimidazole, followed by regioselective nitration by nitric acid, methylation, hydrogenation and upon cyclization with formamide afforded compound **197**. Compound **197** was chlorinated with phosphorus oxychloride and then condensed with piperazine gave compound **198**. Compound **198,** upon treatment with isocyanate, provided compound **185**.

Matsuno *et al.* studied the effect of combination of substituents on the quinazoline ring and *N*-substituted (thio)urea moiety (prototype **XXXIII**) for several earlier known potent analogues like KN1022 (**158**), KN734 (**176**), **199** and **200** [53].

Structure activity relationship of these β-PDGFR inhibitors showed that quinazolines compounds without substitution at C-6 and C-7 positions exhibited less to moderate activity. Quinazoline analogues with mono methoxy group were weaker than dimethoxy analogues. Among the dimethoxy analogues, order of potency is following: C-6 = C-7 >> C-5. Quinazolines possessing C-8 methoxy group had no activity. Polar group and increase in the bulk of halogen at these positions exhibited reduction in activity. In disubstituted (C-6 and C-7 positions) compounds with electron donating group at C-6 exhibited potent activity. Further, addition of group at C-2 and C-8 positions in 6, 7-dimethoxy quinazoline also showed low order of activity.

Compound **201** with C-7 methyl group, however, exhibited the most potent activity with IC_{50} of 0.10 μM/L. While, the C-6 and C-7 disubstituted quinazoline derivatives compounds **202** and **203** showed equally potent activity and was comparable to dimethoxy compounds **199** and **200.**





	R_1	R_2
a	OH	OMe
b	OMe	OH

	R_1	R_2
a	OBn	OMe
b	OMe	OBn

	R_3	R_4
180	OEt	OMe
181	$O(CH_2)_2OMe$	OMe
182	OMe	OEt
183	OMe	$O(CH_2)_2OMe$
184	OMe	$O(CH_2)_2OEt$

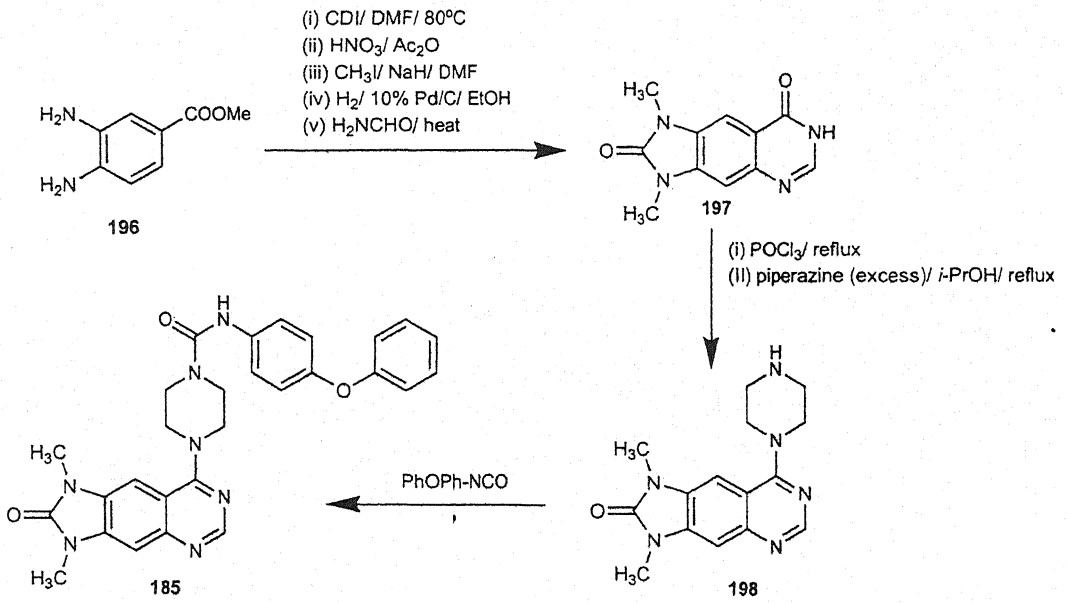
	R_3	R_4
a	OEt	OMe
b	$O(CH_2)_2OMe$	OMe
c	OMe	OEt
d	OMe	$O(CH_2)_2OMe$
e	OMe	$O(CH_2)_2OEt$

Scheme 28.

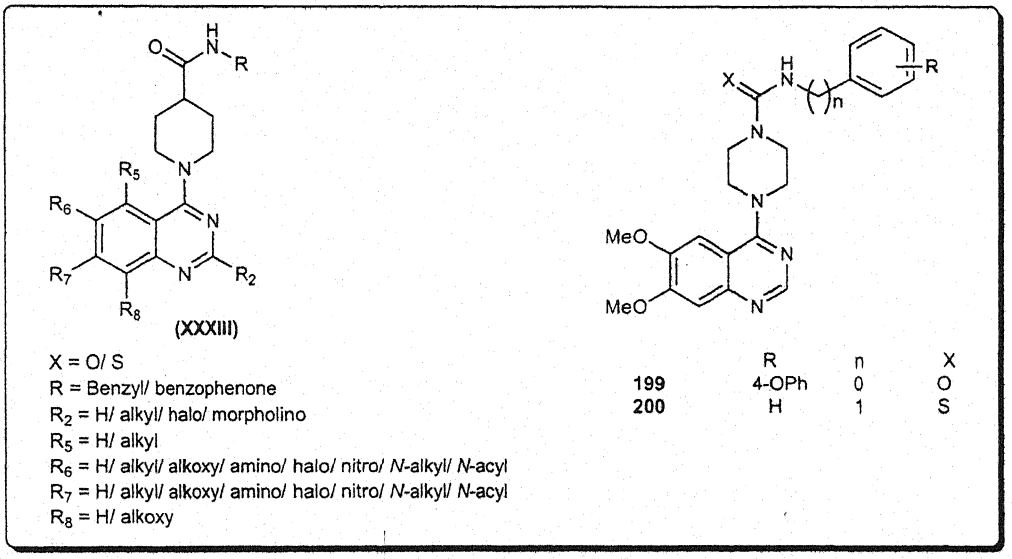
For the synthesis of the potent compounds (**201-203**), two methods were employed as shown in scheme **30** [53]. In the first method (A), the 4-chloroquinazoline (**204**) was condensed with piperazine to give compound **205**, which was then reacted with appropriate iso(thio)cyanate to provide the corresponding derivatives **201-203**. While, in second method (B), the chloroquinazoline (**204**) was reacted with *N*-Boc-piperazine to give compound **206** and which was then deprotected with trifluoroacetic acid and condensed with appropriate isothiocyanate to yield their respective derivatives **201-203**.

The 4-[4-*N*-(substituted-thio-carbomyl-1-piperazinyl]-6,7-dimethoxyquinazoline derivatives [9, 45, 51, 53] were earlier reported as potent and selective β-PDGFR inhibitor. Heath *et al.* carried out the optimization study, in order to improve the potency, pharmacokinetic property and synthesized compounds of prototype **XXXIV** [9].

Structure activity relationship study was carried out by changing the orientation of aryl/heteroaryl ring relative to thiourea and changing the distance between thiourea and its aryl group. Piperidine group on C-7 linker gave rise to more potent compounds. While, the benzyl thiourea derivatives showed enhanced inhibitory activity in both C-7 ethoxy and C-7 propyloxy linker series. Among the different benzyl thiourea derivatives, compound **207** is found as a most potent derivative, which exhibited selectivity for PDGFR



Scheme 29.



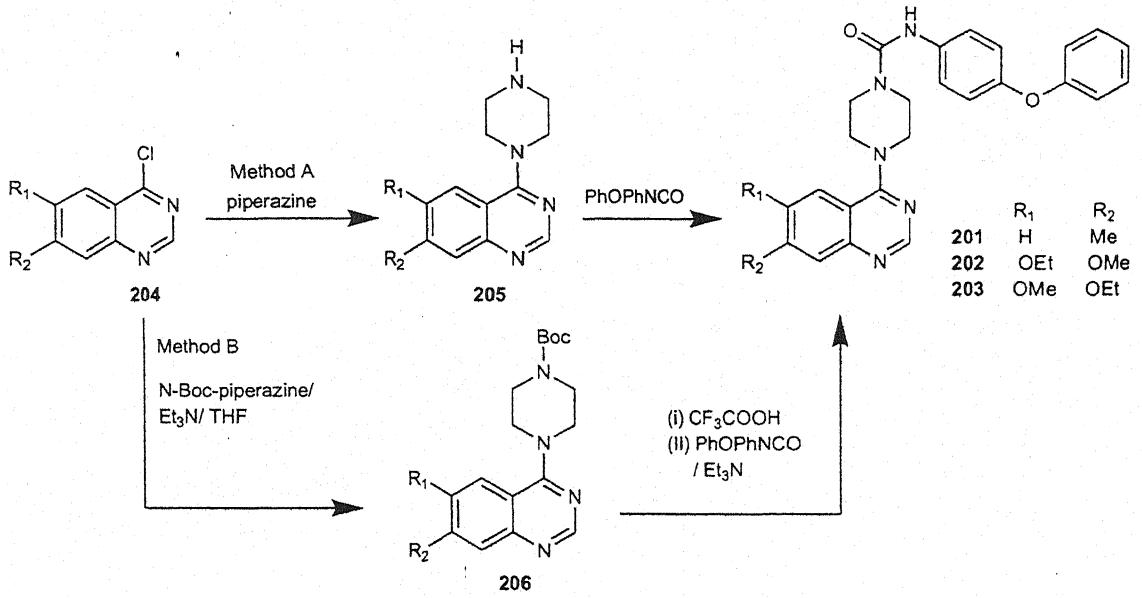
over Flt-3. In case of heterocyclic ring, the electron-withdrawing groups are preferred. The C-3',4'-disubstituted derivative compound **208** retained the activity. Compound **209**, which is a biaryl derivative, is also a potent compound amongst the aryl thioureas derivatives. Cyanoguanidine derivatives of thiourea series were also synthesized but these compounds have diminished order of activity.

Inhibitory activity was checked on the β-PDGFR, c-Kit and Flt-3 and CSF-IR. Compounds **207** (CT53986), **208** (CT53605) and **209** (CT54254) showed IC_{50} value of 61, 129 and 156 nM on MG63 cells respectively.

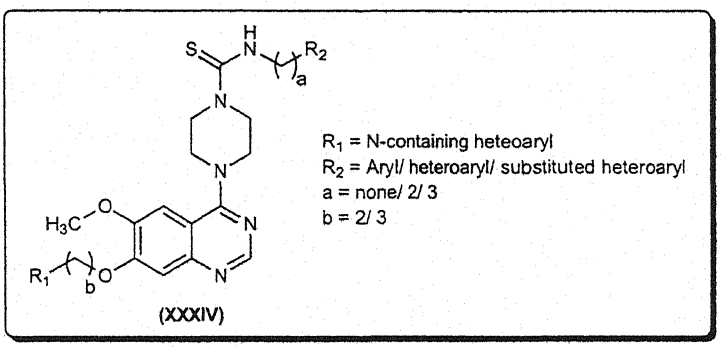
Compounds **207-209** were synthesized as depicted in scheme **31** [9]. Compounds **207-209** could be obtained by three methods: In the first method, compound **211** was reacted with thiophosgene to provide thiochloro derivative **210**, which upon treatment with appropriate amine yielded compounds **207-209**. In the other method, compound **211** on treatment with isothiocyanate afforded the respective thio urea derivatives and which upon *N*-alkylation with appropriate alkylamine furnished the respective potent compounds **207-209**. In an alternate third way, the desired amines were reacted with TCDI (thiocarbonyl diimidazole). The resulting mixture of isothiocyanate/amino thiocarbonyl imidazole was treated with **211** in presence of triethylamine as a base to provide **207-209**.

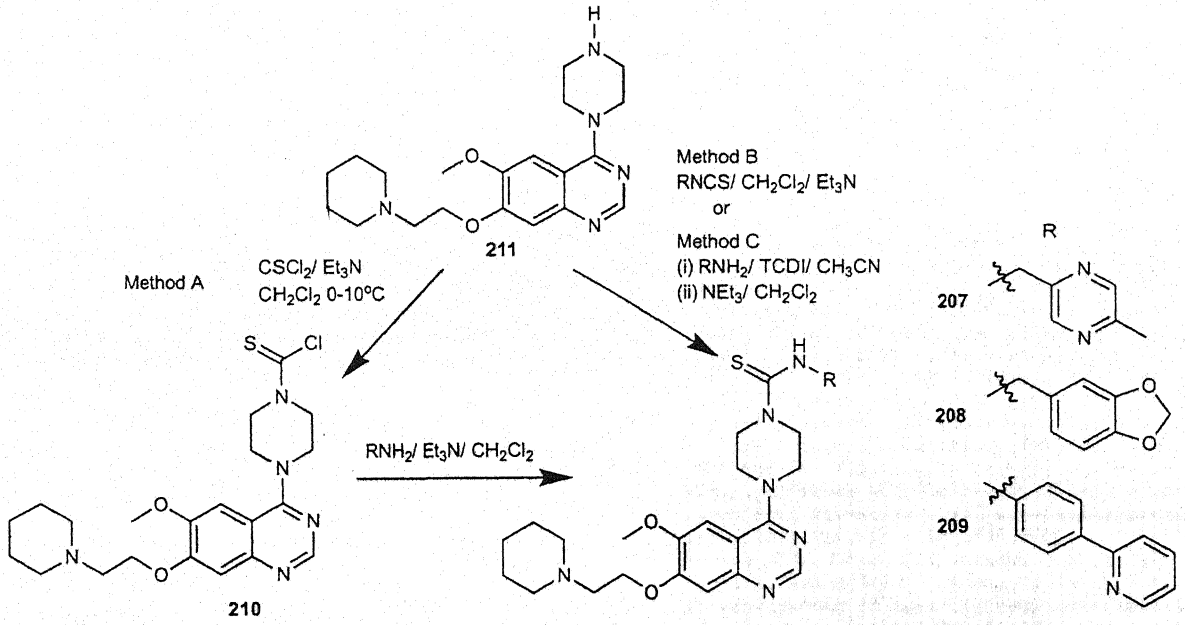
CONCLUSION

In the present review, the discovery and development of quinazolines as tyrosine kinase inhibitors have been systematically discussed. It is evident that certain quinazolines have inhibitory activity against EGFR, VEGFR and PDGFR kinases and that they are active or potential anticancer agents. Three quinazoline derivatives, gefitinib (Irresa), erlotinib (Tarceva), and canertinib, all of which

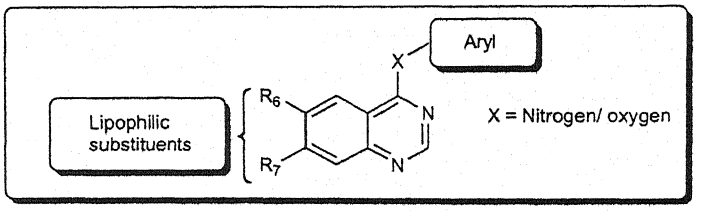


Scheme 30.





Scheme 31.



are tyrosine kinase inhibitors, are now used clinically. Other quinazoline-based derivatives have tyrosine kinase inhibitory activity in the nanomolar range. The structure-activity relationship for quinazolines as tyrosine kinase inhibitors has been established. The C-4, C-6, and C-7 positions are appropriate sites for designing new tyrosine kinase inhibitors. For new compounds, determination of their structure-activity relationships should help the medicinal chemist in designing more effective tyrosine kinase inhibitors.

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Betulinic Acid Derivatives as Anticancer Agents: Structure Activity Relationship

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Abstract: Betulinic acid, a pentacyclic triterpene, is widely distributed throughout the tropics. It possesses several biological properties such as anticancer, anti-inflammatory, antiviral, antiseptic, antimalarial, spermicidal, antimicrobial, antileshmanial, antihelmentic and antifeedent activities. However, betulinic acid was highly regarded for its anticancer and anti-HIV activities. Anticancer role of betulinic acid appeared by inducing apoptosis in cells irrespective of their p53 status. Due to high order safety in betulinic acid, a number of structural modifications carried out to improve its potency and efficacy. The C-1, C-2, C-3, C-4, C-20 and C-28 positions are the diversity centers in betulinic acid, and the derivatives resulted on various structural modifications at these positions screened for their anticancer activity. This review presents the structure activity relationship carried out on C-1, C-2, C-3, C-4, C-20, C-28, A-ring, D-ring and E-ring modified betulinic acid derivatives. We have compiled the most active betulinic acid derivatives along with their activity profile in each series. Structure activity relationship studies revealed that C-28 carboxylic acid was essential for the cytotoxicity. The halo substituent at C-2 position in betulinic acid enhanced the cytotoxicity. Though the relation of the cytotoxicity with the nature of substituents at C-3 position could not be generalized but the ester functionality appeared to be a better substituent for enhancing the cytotoxicity. An interesting observation is that the three rings skeleton (A, B and C rings) had played an important role in eliciting anticancer activity, which could be a new molecular skeleton to design new anticancer drugs.

Key Words: Betulinic acid, Betulinic acid derivatives, Anticancer, Structure activity relationship (SAR).

1. INTRODUCTION

Cancer, a disease of worldwide importance, causes more than five lakhs death annually in only USA and occupies the second rank after heart disease as a killer [1]. Despite the major achievements in different new areas of drug discovery research, the successful treatment of the cancer still remains a significant challenge. The first drug, N-mustard [2] appeared for more than five decades ago. Afterwards, a number of DNA alkylating agents, antimetaboltes and animitotic agents discovered and several of them are still in use for treatment of cancer.

Taxol, a diterpene ester, isolated from *Taxus brevifolia*, is the most promising antitumor agent developed around three decades ago and approved by the Food and Drug Administration (FDA) for the treatment of refractory ovarian cancer and breast cancer. The most leading anticancer product, Paclitaxel (1) of Bristol-Meyers Squibb having recorded sales of $243 million in 2004, is active against a broad range of cancers that are generally considered to be refractory to conventional chemotherapy [3,4]. It binds strongly to tubulin and showed high toxicity against B-16 melanoma [5] and is currently regarded as one of the best anticancer agent. Later, during the development of the taxane derivatives, another potent analog appeared named as docetaxel (Taxotere) (2) [6-8]. Docetaxel is around 2.5 fold more active in causing inhibition of cell replication and acts on S-phase as compared to paclitaxel. Paclitaxel also binds to tubulin and induces its polymerization and promoting stable microtubule formation [9].

Revolutionary discoveries in the field of molecular biology resulted a number of new biological targets such as tyrosine kinases, farnesyltransferases, protein kinases, histone deacetylases, glutathione S transferases and DNA/RNA polymerases, which have been widely used for the designing of new anticancer agents. However, tyrosine kinases have been successfully exploited in cancer chemotherapy and four drugs namely Irresa (3), Tarceva (4), Gleevec (5) and Canertinib (6), as its inhibitors, reached to the market. Moreover, several small synthetic molecules as tyrosine kinase inhibitor are in clinical development *e.g.*, PTK787/ZK 222584 (7) and SU11248 (8) for the treatment of human cancers [10]. Around twenty molecules, as tyrosine kinase inhibitors, are currently in different clinical phases [11]. In addition, approximately twenty-eight molecules, as inhibitors of other targets described above, are at various stages of clinical trials. Despite these discoveries, new approaches required for major improvement of therapeutic agents. Therefore, new broad-spectrum cytotoxic analogs and target specific agents are being continuously synthesized worldwide in order to find anticancer agents with lesser toxicity and more potency as well as efficacy.

2. BETULINIC ACID

Natural products played a major role in the anticancer drug discovery. Over 60% of the anticancer drugs are of natural origin [12]. Betulinic acid (9), 3β-hydroxy-lup-20(29)-en-28-oic acid, a naturally occurring pentacyclic lupane type triterpene, is widely distributed through out the

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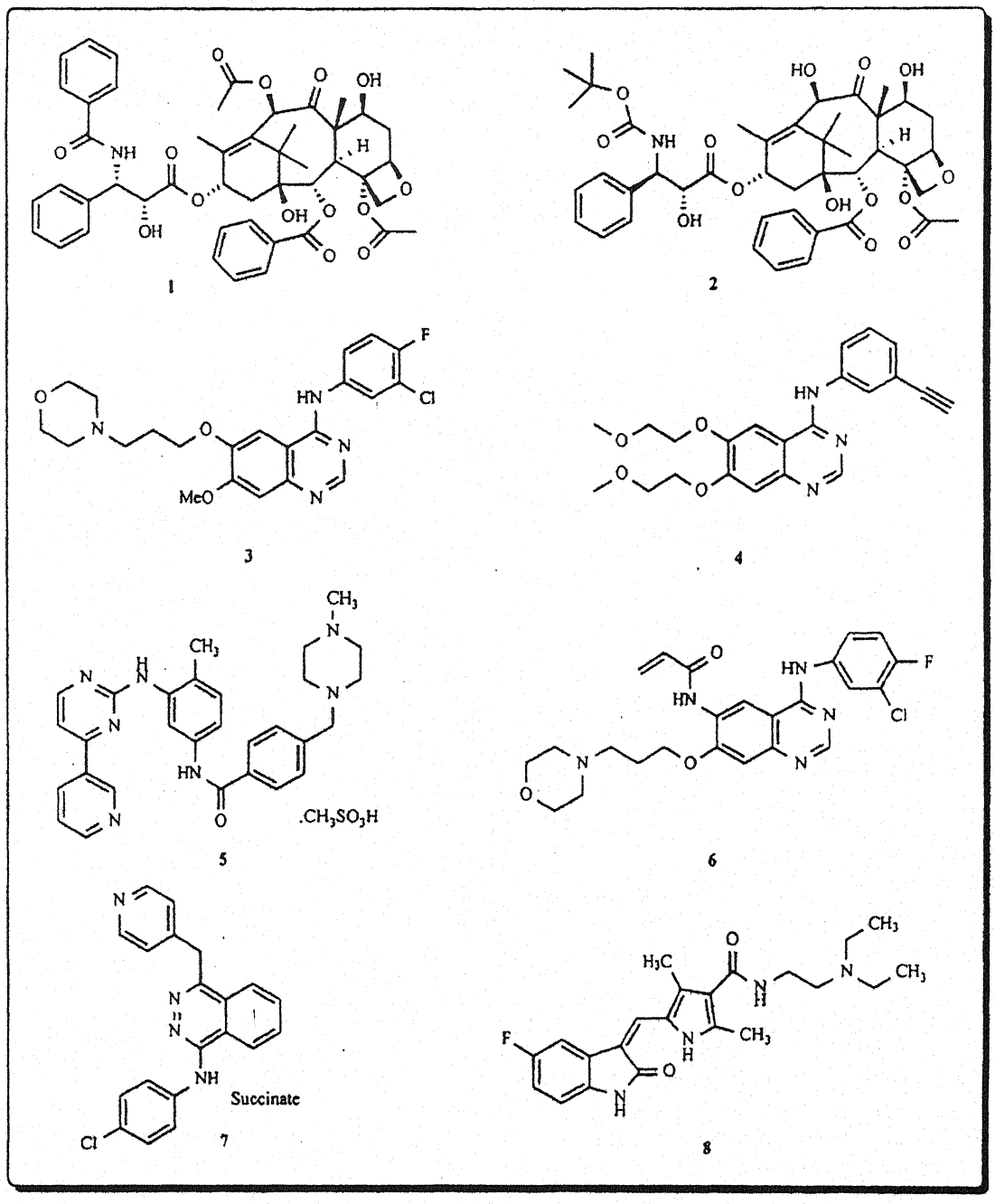


tropics [13]. Betulinic acid is isolated from various plants such as *Tryphyllum peltatum, Ancistrocladus heyneaus, Diospyrus spp.* [14], *Tetracera boliviana, Ziziphus spp.* [15] and *Syzygium farmosanum*, etc. A variety of biological properties ascribed to betulinic acid such as anticancer, antiinflammatory, antiviral, antiseptic, antimalarial, spermicidal, antimicrobial, antileshmanial, antihelmentic and antifeedent activities [16, 17], although the clinical activity has not yet been established.

However, betulinic acid was recognised for its anticancer and anti-HIV activities [18, 19]. Previous reports revealed that betulinic acid is a melanoma specific cytotoxic agent [20]. Recent evidence indicated that betulinic acid also possesses a broader spectrum of cytotoxic activity against other cancer cell lines. Betulinic acid has shown to function through the induction of apoptosis [21-23] irrespective of the cells p-53 and CD-95 status. Some experimental reports indicated that betulinic acid acts through the mitochondrial pathway [24,25], though the precise molecular target and mechanism of action are not yet clear and is now the focus for number of ongoing research programs [26]. As far as toxicity of betulinic acid is concerned, it has been found highly safe even at the dose of 500 mg/Kg body weight. Therefore, these findings and favorable therapeutic index, made betulinic acid a very attractive agent for the clinical treatment for various types of cancers.

The *in-vitro* cytotoxicity activity (IC_{50} values) of betulinic acid has been summarized herein. The IC_{50} values of betulinic acid [27,28] in melanoma cancer cell lines (SKMEL-2, SKMEL-3, UACC257, UISO-Mel-1, UISO-Mel-2 and UISO-Mel-4) are found in range of 0.69 to 4.8 μg/mL, while in breast cancer cell lines (BC1, BT549 and MCF7), IC_{50} values were found >20 μg/mL. In colon cell lines (Caco2, COL2, HCT116, LNCaP and SW620), IC_{50} values of betulinic acid were found in the range of 1.23 to >20 μg/mL. In lung cancer cell lines (LNCaP, A549 and LU1), IC_{50} value were in the range of 7.7 to >20 μg/mL, while in giloblastoma cell lines (A172, SK14, SK17, SK19, SK22, SK37, SK51, SK55, SK60, U87MG, U118, U138, U251, U343 and U373), it was found in the range of 5 to >20 μg/mL. In medulloblstoma cancer cell lines (D283, D341, Daoy, MEB1, MHH1, MHH3 and MMH4), IC_{50} values were lying in between 3 to 15 μg/mL and in ovarian cancer cell lines (A2780, IGROV1 and OVCAR5), it varies from 1.8 to 4.5 μg/mL. In prostate cancer cell lines namely PC3 and KB, IC_{50} value was 1.9 and >20 μg/mL, respectively. It was, therefore, concluded that betulinic acid is a promising anticancer agent. And as a result, further studies have been performed to synthesize betulinic acid analogs in an effort to establish a meaningful structure activity relationship as well as to get more potent anticancer agents.

3. STRUCTURE ACTIVITY RELATIONSHIP (SAR) FOR BETULINIC ACID DERIVATIVES

The C-1, C-2, C-3, C-4, C-20, C-28, A-ring, D-ring and E-ring are the positions for diversification in betulinic acid. Hundreds of derivatives prepared and tested for their cytotoxic activity. Herein, the structural modifications made at these positions in betulinic acid as well as their affect on the cytotoxicity, is compiled. We have assembled the active betulinic acid derivatives along with their cytotoxicity profile in each series.

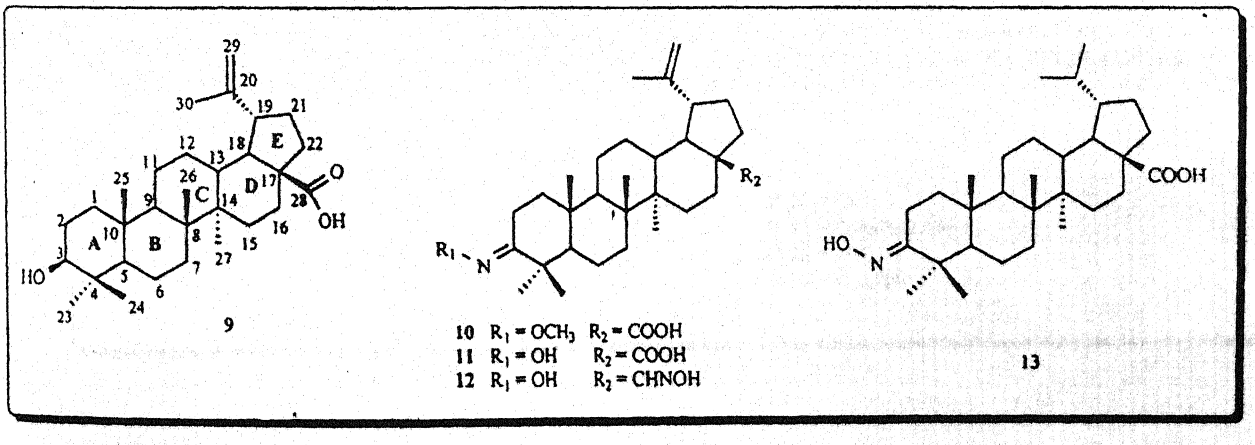
3.1. Modifications at C-1, C-2 and C-3 in Betulinic Acid

The C-3 position in betulinic acid was widely exploited and hundreds of derivatives such as oxime, O-acyl, benzylidenes, hydrazine and hydrazones have been reported in the literature. A brief SAR in each of these series of compounds has been described herein. Kim *et al.* [29] synthesized different C-3 oxime derivatives of betulinic acid (*e.g.*, compounds **10-12**). The loss of the activity was, however, observed in these compounds against MEL-2 cell line but exhibited interesting activity in KB cell line. Compound **12** was active against KB cell line (ED_{50} = 3.3 μg/mL), whereas slightly less active (ED_{50} = 2.2 μg/mL) on MEL-2 cell line than betulinic acid (ED_{50} = 1.2 μg/mL). In general, hydrogenation at C-20 position in C-3 modified derivatives does not show major difference in the anticancer activity in comparison to the corresponding non-hydrogenated molecules. Replacement of the C-28 carboxylic group by either oxime or alcohol or aldehyde or ester resulted loss in the activity. It seems that the free carboxylic at C-28 position is essential for eliciting cytotoxicity in C-3 oxime derivatives.

It is reported that betulinic acid inhibited one of the endogenous factor aminopeptidase-N and mitochondrial function in endothelial cells [30]. Based on these observations, different C-3 modified betulinic acid derivatives synthesized as antiangiogenic agents by Mukherjee *et al.* [31]. Starting from 20, 29-dihydrobetulinic acid, hydroxyl oxime derivative **13** was obtained, which was found to be one of the most potent molecule against lung cancer cell lines such as L132 and A549 with ED_{50} of 1.5 and 1.8 μg/mL, respectively. Compound **13** exhibited an ED_{50} of 1.1 μg/mL against DU145 cancer cell line and is comparable to betulinic acid (ED_{50} = 1.13 μg/mL). As discussed earlier for anticancer activity, in this case also, C-28 carboxylic acid appeared to play an important role in antiangiogenic activity in C-3 oxime derivatives.

A number of 3-*O*- acyl derivatives (**14-17**) prepared by Mukherjee *et al.* [32], The 3-*O*-acyl betulinic acid derivative **14** showed IC_{50} 0.9 μg/mL on ECV304 (endothelial cell line) and low to moderate ECS (endothelial cell specificity). The 3-*O*-acyl dihydrobetulinic acid derivatives, **15** and **16**, exhibited better cytotoxicity (IC_{50} ~ 0.7 μg/mL) in comparison to betulinic acid (IC_{50} ~1.2 μg/mL) on ECV304 cell line. These compounds also showed slightly better ECS values than betulinic acid. Compound **16** showed better inhibition of TLS (tube like structure) formation than betulinic acid.

3-*O*-acetyl 20,29 dibromobetulinic acid (**17**) obtained upon the bromination of the acetylated betulinic acid, showed better activity (ED_{50} = 0.3 μg/mL) against U937 human histocytic lymphoma in comparison to betulinic acid



9

10 R_1 = OCH_3 R_2 = COOH
11 R_1 = OH R_2 = COOH
12 R_1 = OH R_2 = CHNOH

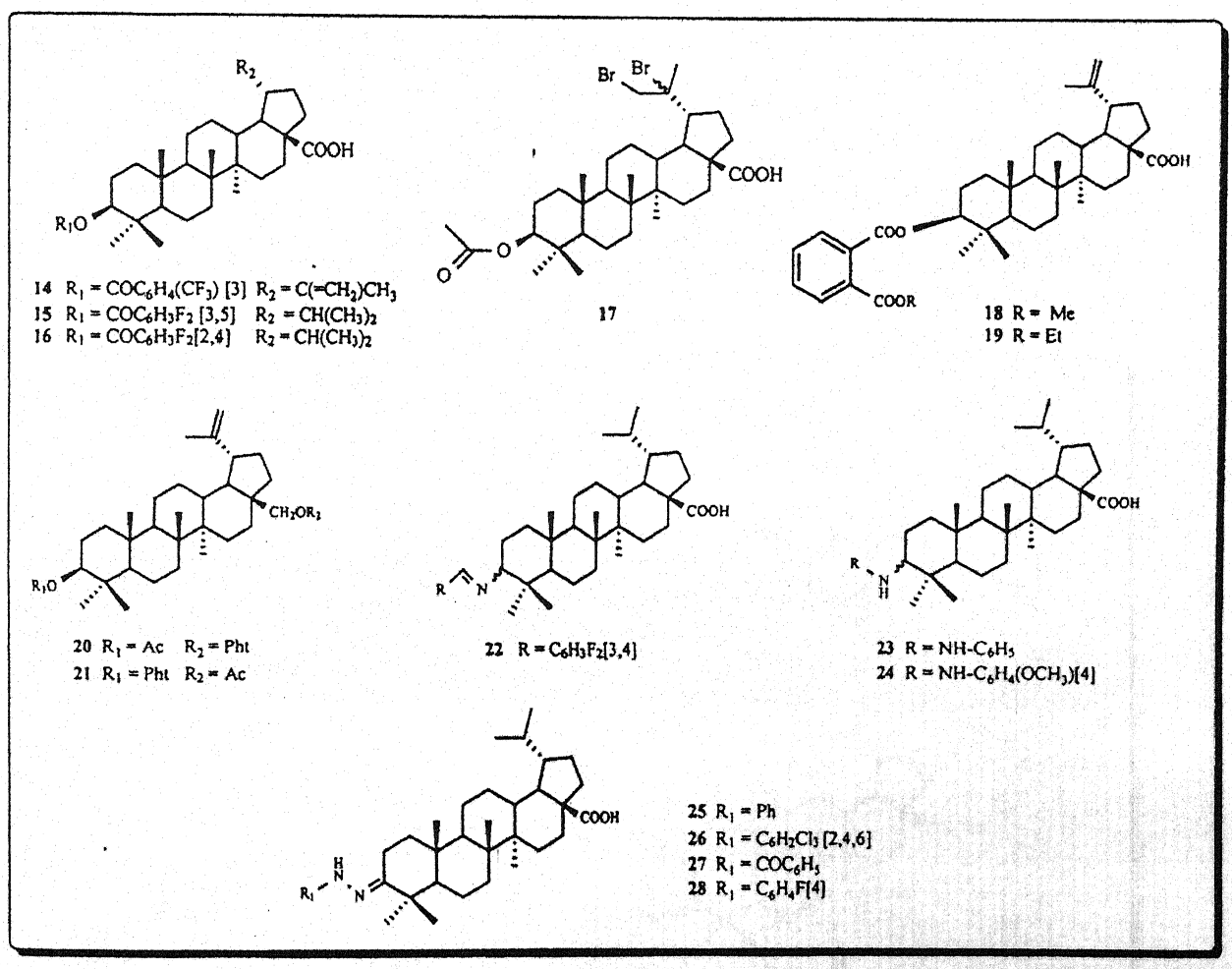
13

(ED_{50} = 1.6 μg/mL). Thus, the bromo substituent at C-20 position in 3-*O*-acyl betulinic acid derivatives played an important role in eliciting cytotoxicity [31].

Based on the structural similarity between betulinic acid and betulin, Kvasanica *et al.* [33] recently prepared different 3-*O*-phthalic ester derivatives (**18-21**). Several of them found to be more cytotoxic and polar in comparison to betulinic acid. Compounds **18** and **19** were prepared by transfer-hydrogenolysis of hemiphthalate of benzyl ester of betulinic acid. Both compounds **18** and **19** showed activity against a number of cancer cell lines like CEM, K562, K562-tax, HT29, PC-3 and SK MEL2. Compound **18** was found as a most active compound in this series with IC_{50} of 5.7, 8.8, 7.5 μg/mL on CEM, K562 and HT29 cancer cell lines, respectively, in comparison to betulinic acid (IC_{50} = 27.5, 54.8, 84.5 μg/mL in the same cell lines, respectively). Betulin that is inactive in nature, but its betulin phthalate ester derivatives **20** and **21** have shown good activity. Compound **20** obtained by mild basic hydrolysis of betulin diacetate, whereas **21** was prepared by selective acetylation of primary hydroxy group of betulin. Compounds **20** and **21** showed IC_{50} = 8.3 and 34.2 μg/mL, respectively, on K562-tax cancer cell line in comparison to betulinic acid (IC_{50} = 108.2 μg/mL). From SAR of these compounds, it was concluded that derivatization of hydroxy group at C-3 in betulinic acid by phthalic anhydride enhanced the cytotoxic activity. In particular, electron withdrawing group in the aromatic ring in 3-*O*-acyl group have enhanced the cytotoxicity with low to moderate ECS value, while bulky and electron donating group lowered the activity.

A number of benzylidene (*e.g.*, **22**), hydrazine (*e.g.*, **23** and **24**) and hydrazone (*e.g.*, **25-28**) derivatives of betulinic acid have been reported [31,32]. The 20, 29-dihydro-3 benzylidene betulinic acid derivative (**22**) was found highly cytotoxic (IC_{50} ~ 0.35 μg/mL) and showed good anti TLS property. Hydrazine derivatives, **23** and **24**, exhibited high cytotoxicity activity (IC_{50} ~ 0.5 and 0.4 μg/mL) on ECV304 cell line and high ECS value. It can be predicted that the introduction of electron donating group in aromatic ring at C-3 hydrazine functionality in 20, 29-dihydrobetulinic acid improved the activity. From SAR, it was concluded that protection of C-28 carboxylic acid in this series of betulinic acid or in dihydrobetulinic acid derivatives lowered the cytotoxicity.

The reaction of dihydrobetulinic acid with phenyl hydrazine furnished corresponding 3-phenyl hydrazone derivatives (*e.g.*, **25-28**). Compound **25** showed high order activity against DU145 and PA-1 cell lines with ED_{50} of 0.6 and 0.4 μg/mL, respectively, in comparison to betulinic acid (ED_{50}



of 2.6 and 4.1 μg/mL) [31]. Compounds **26-28** showed IC_{50} < 0.4 μg/mL on ECV cell line with moderate to high ECS value (ECS >10) against A-549 cell line. These betulinic acid derivatives inhibited around 13.1-49.2% of tube like structure formation (TLS) of ECV304 cells in a matrigel tube formation assay in comparison to 5.5% of betulinic acid [26]. It seems that hydrazone group at position-3 together with 20,29 dihydro moiety played a key role in endothelial cytotoxicity preferably in anti-TLS activity.

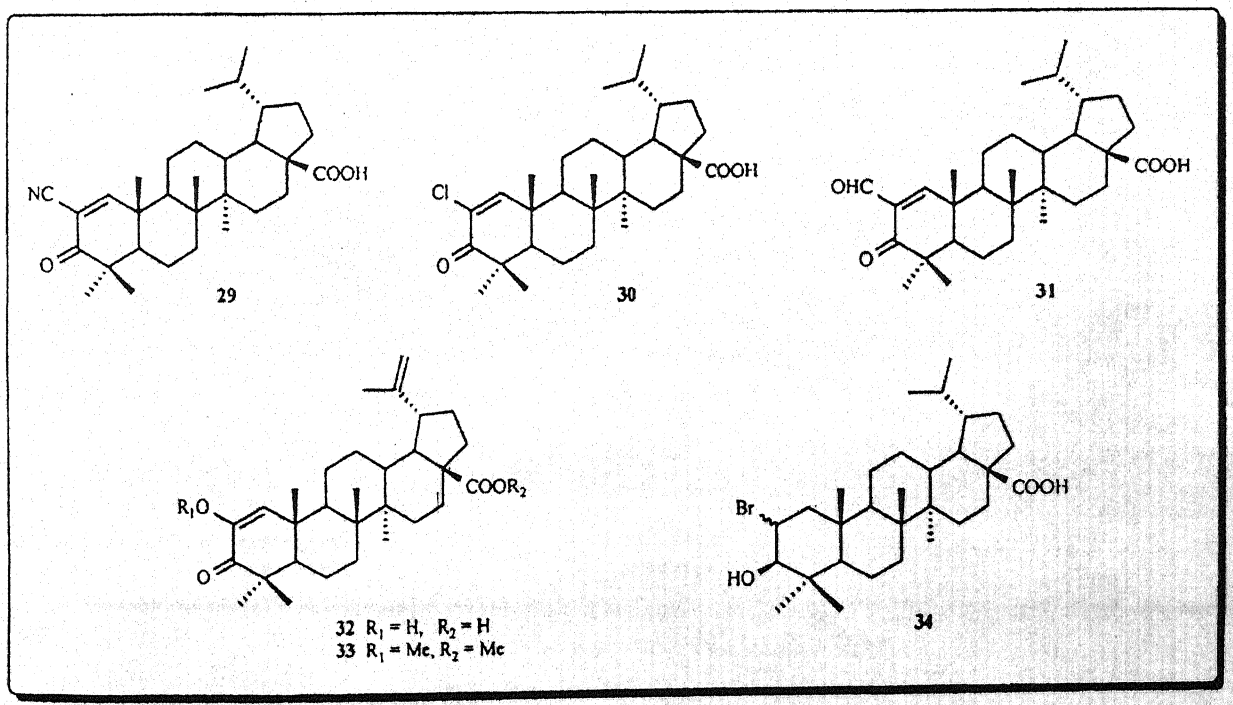
You *et al.* [34] synthesized several C-1 and C-2 modified betulinic acid derivatives (*e.g.*, **29-31**) in which A ring is substituted by different electron-withdrawing group with least steric nature. For example, 3-*O*-1-ene moiety (in A ring) was introduced along with different groups at C-2 position, such as 2-cyano (compound **29**), 2-chloro (compound **30**) and 2-formyl (compound **31**). The synthesis of compounds **29-31**, were performed in the following ways: betulonic ester was converted to 20-hydromethylene-3-oxo derivative followed by isoxazole ring formation and which upon ring opening and oxidation of the product thus obtained, furnished compound **29**. The 2-chloro (compound **30**) was obtained by converting C-28 protected dihydrobetulonic acid to an epoxy derivative followed by the acid-base treatment. Compound **31** was synthesized by converting 3-oxo-betulinic acid to hydroxymethylene derivative followed by subsequent oxidation and base treatments. These compounds were tested on different cell lines such as SK-MEL-2, A-549 and B16-F10 for their anticancer activity. Compound **30** was found most active in the series, as it was around 59 fold more potent than betulinic acid (ED_{50} = 0.13 μg/mL) on SK-MEL-2 cell line. Compound **29** exhibited higher cytotoxicity (ED_{50} = 0.81 μg/mL) in comparision to betulinic acid (ED_{50} = 7.62 μg/mL) on SK-MEL-2 cell line. Compound **31** (ED_{50} = 0.26 μg/mL) elicited 28 times higher efficacy than betulinic acid. It was interesting to note that in this case, double bond at C-20 position was not essential for cytotoxicity activity. The C-28 carboxylic acid was, however, critical for the cytotoxic activity, but in few cases methyl ester also found active. Thus, it is predicted that the derivatives with C-28 carboxylic acid and electron withdrawing at C-2 would show strong cytotoxicity.

Urban *et al.* [35] synthesized several diosphenols and seco derivatives of betulinic acid (*e.g.*, **32** and **33**). For the synthesis of diosphenol derivative (**32**), betulinic acid was oxidized to 3-*O*-betulinic acid, which upon further oxidation provided compound **32**. Dimethylation of compound **32** furnished **33**. Cytotoxicity of these compounds were checked in several cell lines such as CEM, HT 29, K562, K562 Tax, PC-3, A549, DU 145 MCF 7 and SK-Mel2. Both the compounds **32** and **33** were highly cytotoxic on CEM cell line with IC_{50} of 4 and 5 μmol/L, respectively, in comparison to betulinic acid (IC_{50} = 27 μmol/L).

Very few anticancer compounds are known to be active against the tumour cell, endothelial cells and simultaneously act as angiogenesis inhibitors. Mukherjee *et al.* [26] synthesized several 2-bromo derivatives, which exhibited potent activity on endothelial cells as well as tumor cells. For example, compound **34** was highly potent (IC_{50} ~ 0.27 μg/mL) on ECV 304 cell line and having moderate ECS value as compared to betulinic acid (IC_{50} ~ 1.26 μg/mL). In general, the halo substituent in C-2 modified derivatives have improved the cytotoxicity.

3.2. Modifications at C-1, C-2, C-3 and C-4 in Betulinic Acid

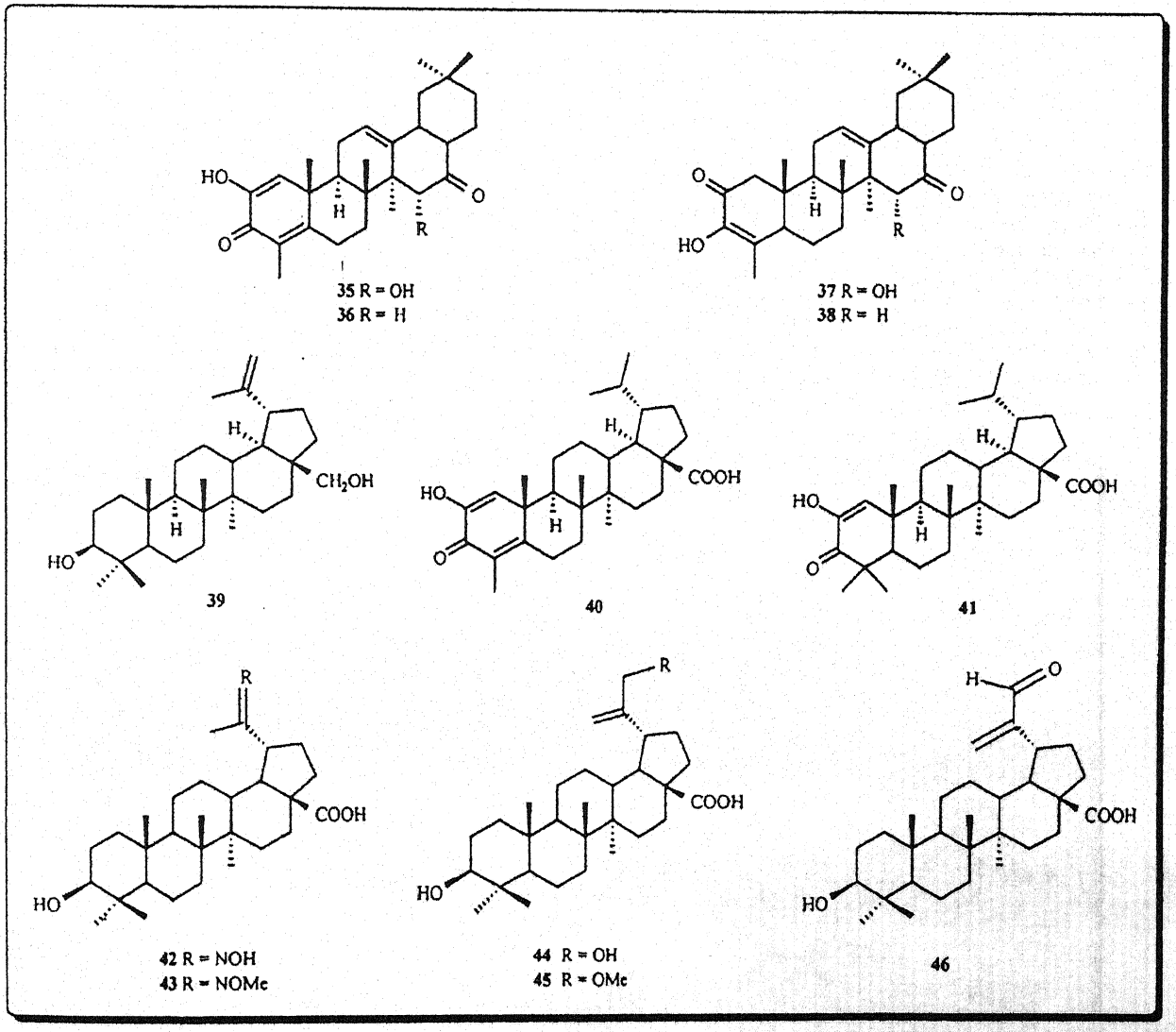
Several C-1, C-2, C-3 and C-4 modified betulinic acid derivatives synthesized and screened for cytotoxicity and the SAR results are summarized here. Remangilones (**35-38**) are the cytotoxic compounds obtained from the plant *Physena*



medagascariensis. Deng *et al.* [36] converted betulin (compound 39) into the 24-nor analogous (compound 40), possess the same ring system as in remangilones. Compound 40 was synthesized in the following manner. Ring A of betulin was opened under harshed condition using Suarez cleavage and an extra carbon at C-24 position was then removed by oxidative cleavage. The A ring reclosure was carried out by SmI_2 mediated pinacol coupling to afford compound 40. Compound 41 was prepared by the oxidation of hydrogenated betulin and followed by its conversion to methyl ester and then silylation and demethylation. Both these compounds (40 and 41) were screened for their biological activity and calculated GI_{50}, TGI and LC_{50} values. These compounds were found slightly less active in comparison to betulinic acid. Compound 40 showed GI_{50} value < 10 nM on a particular ovarian cancer cell line (SK-OV-3). Whereas, 41 exhibited GI_{50} activity in range of 2-7 μM activity on six leukemia cell lines.

3.3. Modifications at C-20 in Betulinic Acid

Kim *et al.* [37] carried out several modifications at alkene group at C-20 position in betulinic acid (*e.g.*, compounds 42-45). This position was found to be sensitive to size and electron density of the substituents in retaining the cytotoxicity. The chemical modification at C-20 was carried out by converting it into ketone followed by the treatment with different hydroxylamines to get corresponding oximino derivatives (compounds 42 and 43). Oximes appeared to result in loss of cytotoxicity. Also upon reduction of keto group to secondary alcohol resulted to an inactive compound. Other derivatives like compounds 44 (primary alcohol) and 45 (methoxy ether) were synthesized by converting C-29 position of 3-acetybetulinic acid to bromo derivative and which, upon further reaction with desired substituent followed by hydrolysis provided the required products. Compounds 44 and 45 found less active than betulinic acid on HCT-116 and M14-MEL cell lines. Sarek *et al.* [38] synthesized a new class of betulinic acid derivatives (46) called Betulinines in which C-20 position was modified with different oxygen containing functions. Compound 46 showed TCS_{50} (concentration with 50% tumor cell survival) 4.0 μM on CEM cancer cell line. This compound also showed accumulation of cells in G2/M and S region of cell cycle. The above result showed the role of size and electrostatic effect



at C-20 position in betulinic acid. Therefore, C-20 was found to be undesirable position in betulinic acid for derivatization. However, the cytotoxic activity of the compound **46** is assigned due to the presence of α-unsaturated keto group.

3.4. Modifications at C-28 in Betulinic Acid

As discussed earlier, betulinic acid displayed a good anticancer activity but problem associated with its water solubility. Jeong *et al.* [39] produced the C-28 amino acid conjugates (**47-50**), which caused improvement in selective toxicity as well as water solubility of betulinic acid. Activity of the compounds checked in human melanoma (MEL-2) and fibrosarcoma (KB) cell lines. In case of alanine methyl ester (compound **47**), ED_{50} was 3.5 μg/mL on MEL-2 cell line while its free acid, compound **48**, was found effective against both MEL-2 and KB cell lines with ED_{50} of 1.5 and 4.6 μg/mL, respectively. Compound **48** was found to be better in comparison to betulinic acid (ED_{50} = 4.2 and >20 μg/mL in the same cell line, respectively) and simultaneously, improved the water solubility. The free acid of glycine (compound **49**) was effective on MEL-2 cell line similar to betulinic acid with ED_{50} of 4.2 μg/mL but showed the best water solubility (2 mg of **48** was dissolved in 200 μL of DMSO and upon dilution of 20 μL of this solution with distilled water provided water solubility of 100x). ED_{50} of methyl ester of valine (compound **50**) was found to be 2.1 μg/mL. It is observed that glycine conjugate of betulinic acid had improved the solubility without affecting the cytotoxicity.

Ramadoss *et al.* [40,41] synthesized various C-28 ester derivatives of betulinic acid, and in general, cytotoxicity did not improve. However, compounds **51** and **52** showed selective cytotoxicity on PA-1 (human ovary) cancer cell line with ED_{50} of 3.1 and 1.3 μg/mL, respectively, better than betulinic acid (ED_{50} >10 μg/mL).

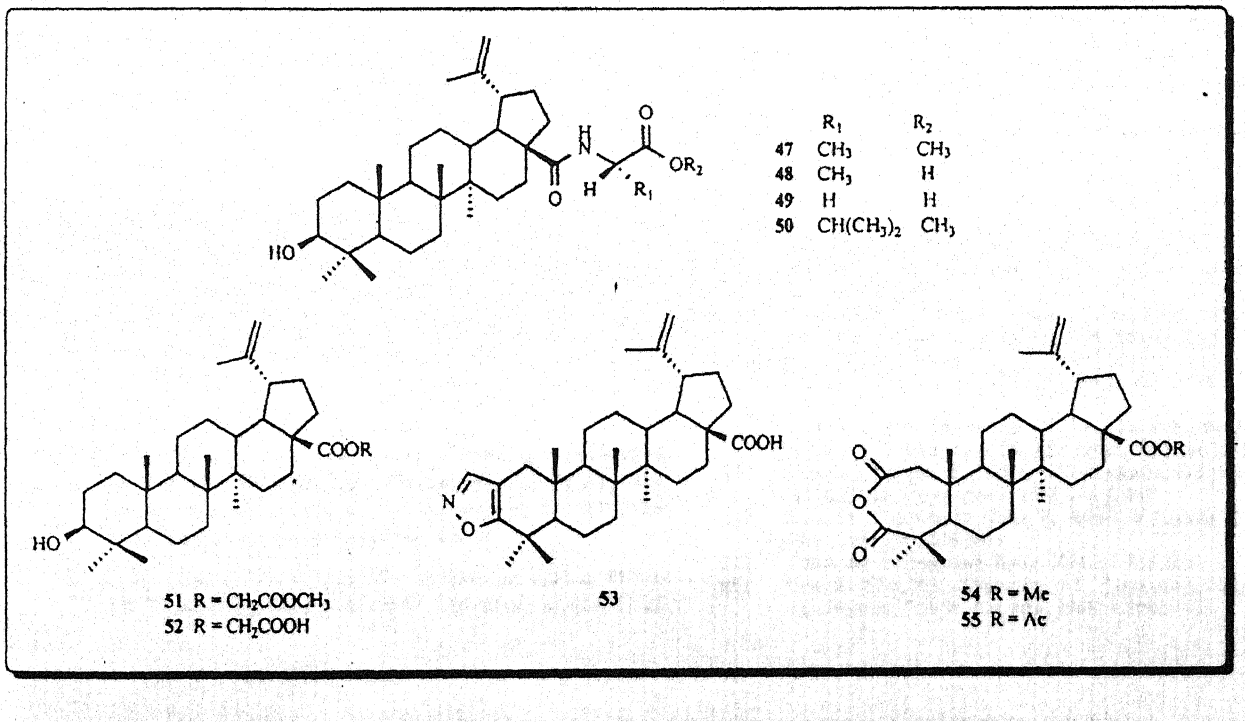
3.5. Ring A Expansion in Betulinic Acid

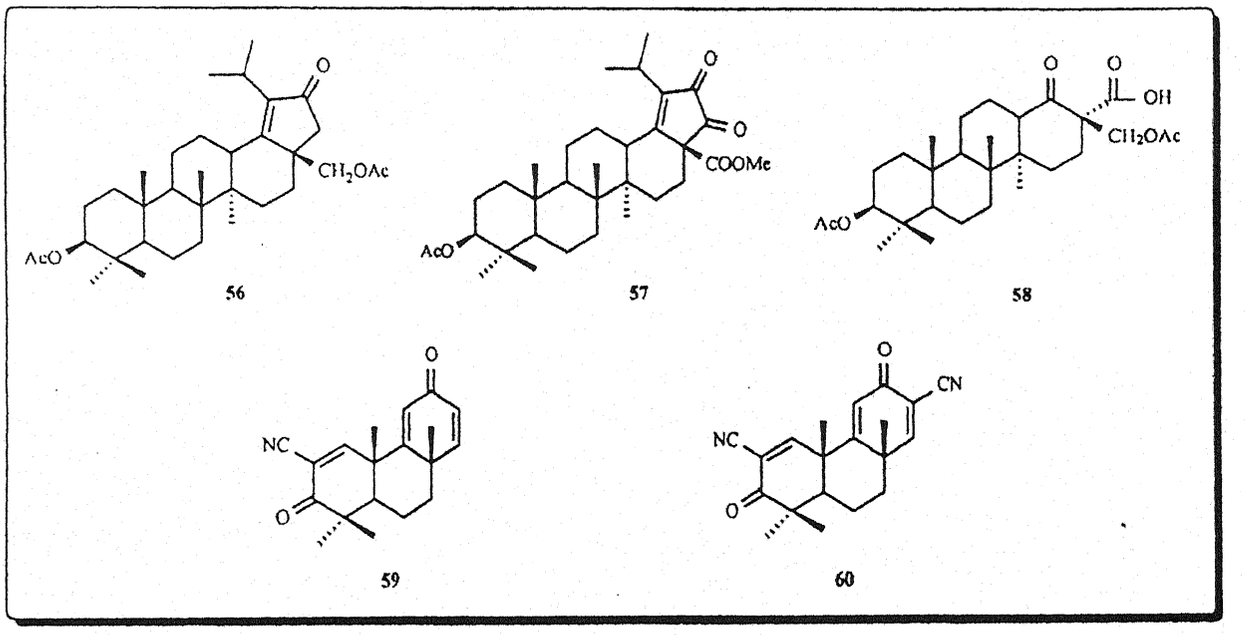
Isoxazole fused (**53**) and seven membered (**54** and **55**) derivatives prepared and screened for the cytotoxic activity. You *et al.* [34] synthesized compound **53** by converting 3-oxo betulinic acid into hydroxymethylene derivative, which upon condensation provided isoxazole ring. This compound showed good cytotoxicity against A-549 cell line with ED_{50} of 1.54 μg/mL, which is around 5 times more than betulinic acid.

Urban *et al.* [35] synthesized seco derivatives (**54** and **55**) of betulinic acid. After converting betulinic acid to betulonic acid, the latter was oxidized to give diosphenol compound, which was then cleaved to seco derivatives and changed to seven membered cyclic anhydrides (**54** and **55**). Compounds **54** and **55** showed the maximum activity on CEM cancer cell line with IC_{50} of 7 μmol/L and 6 μmol/L, respectively, in comparision to betulinic acid (IC_{50} 27 μmol/L).

3.6. Ring E Modifications in Betulinic Acid

Sarek *et al.* [38] modified E ring in betulinic acid with different oxygen functions (compounds **57** and **58**), which were given a common name betulinines. Starting compound **56** (21-oxolup-18-ene-3β, 28-diyl diacetate) was obtained by various chemical modifications of betulin. Compound **56** upon selective saponificatin and oxidation, gave rise to diketone compound **57**. Compound **56** upon oxidation and breaking of the 18,19 double bond gave rise to a tetraketone, which upon further cleavage afforded carboxylic acid derivative **58**. Anticancer activities of these compounds were tested on CEM, epithelial, neuroectodermal and mesodermal tumor cell lines. Apoptosis in CEM cell line was shown by flow cytometry and scanning electron microscopy techniques. Both the compounds **57** and **58** showed, TCS_{50} of 4.4 and 1.0 μM, respectively on CEM cell line. Compound **58**





was found as the most active compound in this series. These derivatives possess broad-spectrum anticancer potency. This compound (**58**) was effective in both drug resistant and drug independent cell lines. Therefore, these molecules may show a good scope for cancer chemotherapy resistant persons. Acetoxymethyl group at C-17 position in compound **58** provided stability due to its steric nature and prevents formation of six membered ring.

3.7. D and E Rings Modifications in Betulinic Acid

Favaloro *et al.* [42] synthesized tricyclic compound **59**, which showed significant inhibitory activity (IC_{50} ~ 0.01 μM) on nitric oxide synthase inhibition, is related to anticancer activity. Further modification of this compound (**59**) was carried out by induction of electron withdrawing group at C-13 position gave rise to a more potent compound **60** (IC_{50} ~ 2.1 nM). This indicated that these tricyclic molecules might provide a new molecular skeleton to design broad-spectrum anticancer agents. The low molecular weight compounds are always preferred in clinics.

4. CONCLUSION

Betulinic acid is a promising new chemotherapeutic agent for the treatment of multiple forms of cancers. Several structural modifications have been carried out at C-1, C-2, C-3, C-4, C-20, C-28, A-ring, D-ring and E-ring in betulinic acid and revealed the several information which are as follows:

1. The halo sustituent at C-2 position in betulinic acid improved the cytotoxicity.
2. Modifications at C-3 position in betulinic acid seemed to be critical. Though, the relation of cytotoxicity and sustituent at C-3 could not be generalized but the ester functionality was found relatively better.
3. C-28 carboxylic acid position in betulinic acid is essential for eliciting the cytotoxicity. Moreover, several C-28 amide conjugate enhanced the solubility of betulinic acid without changing the cytotoxicity profile.
4. The double bond in between C-20 and C-29 position is not good for the derivatisation.
5. Expansion of ring A did not make a major difference in the cytotoxicity. However, since there are only a few reports on synthesis of heterocyclic at ring A, so a detailed investigation is still required to ascertain the actual affect of heterocyclic on the cytotoxicity.

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Amidation of carboxylic acid using EDC; amide

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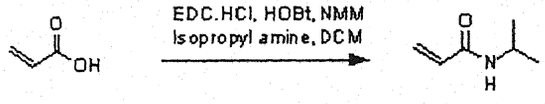
A contribution from the Chemical research , Dabur Group, Dabur



Chemicals Used

Acrylic acid (S. D. Fine Chem) Isopropyl amine (S. D. Fine Chem) 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC.HCl) (Lancaster) 1-hydoxybenzotriazole (HOBt) (SRL) N-methylmorpholine (NMM) (Aldrich) Dichloromethane (Qualigens)

Procedure

A solution of isopropyl amine (4.9 g, 83 mmol) in DCM (20 ml) was added to a stirred and cooled (0°C) solution of acrylic acid (5 g, 69 mmol) in DCM (30 ml) and the mixture was stirred for 5 min. To the resulting reaction mixture HOBt (3.75 g, 28 mmol) and NMM (N-methyl morpholine) (8.4 g, 83 mmol) were added. After 30 min., EDC (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide) (15.9 g, 83 mmol) was added to reaction mixture and stirred for 3 h at 0°C and then at rt for 4h. The mixture was diluted with water; organic layer was extracted and concentrated in vacuum. Crude product was chromatographed on a silica gel (100-200 mesh size) column using MeOH/DCM (2:98) as eluent to provide the

product as buff white solid. The product was further crystallized in hexane. Needle shaped white crystals of the title compound (4.2 g, 54 %) were obtained.

Author's Comments

EDC is one of the mild reagent to synthesize amides. Same procedure can also be used to synthesize esters.

Data

m.p. 60-61oC; 1H NMR (300 MHz, CDCl3): 6.27 (1H, dd, J = 1.6, 16.9 Hz), 6.10 (1H, dd, J = 10.3, 16.9 Hz), 5.72 (1H, bs), 5.61 (1H, dd, J = 1.6, 10.3 Hz), 4.17 (m, 1H), 1.23 (3H, s), 1.19 (3H, s). MS (ES+) m/z (relative intensity) 114 (M+H) (100), 136 (M+H+Na) (50); HPLC purity = 100 %.

Lead Reference

Chan, L.C.; Cox, B. G. J. Org. Chem. 2007, 72, 8863-8869.

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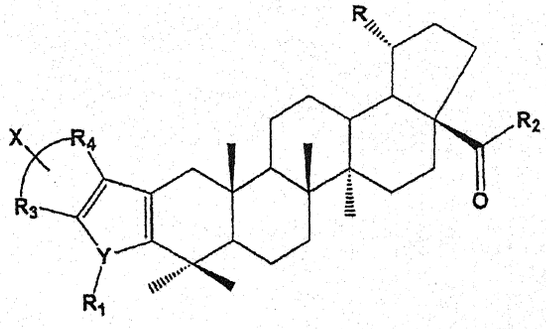
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT,

[Continued on next page]

(54) Title: NOVEL BETULINIC ACID DERIVATIVES



(I)

(57) Abstract: The invention relates to novel betulinic acid derivatives of formula (I), wherein R is C (=CH_2) CH_3 or $CH(CH_3)_2$; R_2 together with the adjacent carbonyl group forms a carboxylic acid, carboxylic acid ester or amide or substituted amide; R_3 or R_4 are hydrogen or aryl with the proviso that both are not independently hydrogen or alkyl or R_3 and R_4 are combined together to form an aryl ring optionally substituted with a group X, wherein X is selected from halogen, alkyl, cyano, nitro, alkoxy, amino or substituted amine; Y is N or O; and R_1 is zero when Y is O, and R_1 is hydrogen, alkyl or aryl alkyl when Y is N, useful for inhibition of tumor cancer cells. Formula (I)

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RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

Published:

— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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Our ref: SS/GD/SNP 3108

05 December 2005

DABUR RESEARCH FOUNDATION
22 Site IV, Sahibabad,
Ghaziabad – 201 010
UTTAR PRADESH
(Facsimile: 95120 2777303/2777753)

Attn: Dr. R. Sankaran
Head – IPR

Dear Sirs,

Re: **DABUR PHARMA LIMITED**
Indian Patent Application **No. Awaited**
Filed on : 29 November 2005
(Novel 1,8-naphthyridine derivatives as anticancer agents)

In accordance with your instructions, we confirm having filed this provisional application on 29 November 2005.

The official filing receipt shall be forwarded to you as soon as it is received from the Patent Office.

The **due date** for filing a complete specification is **29 November 2006**. This date is **not extendible.**

In case you wish to file any corresponding foreign application or a PCT International Application with the benefit of priority date, it must be filed by the **deadline** of **29 November 2006.** This date is **not extendible**.

We look forward to your instructions on the above matter before 1 August 2006.

We also enclose herewith application Form 1 in duplicate for signatures by the inventors. Please return the duly executed Form 1 as soon as possible, preferably by **15 January 2006**.

Also enclosed is a Power of Authority in our favour for due execution and return to us.

For our services and disbursements in preparing and filing this application, our debit note is enclosed.

With best regards,

Yours sincerely,

Sunil Singh

Enclosures:
Debit Note
Form 1 in duplicate
Power of Authority
Specification as filed

FORM 1 THE PATENTS ACT, 1970 (39 OF 1970) & The Patents Rules, 2003 APPLICATION FOR GRANT OF PATENT (See Sections 7, 54 & 135 and Rule 20(1)	(FOR OFFICE USE ONLY) Application No.: Filing Date: Amount of Fee Paid: CBR No.: Signature:

1. APPLICANT(S)

Name	Nationality	Address
DABUR PHARMA LIMITED	INDIAN	3, Factory Road, Adjacent Safdarjung Roa New Delhi – 110 029, India;

2. INVENTORS

Name	Nationality	Address
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[ii] SRIVASTAVA, Sanjay Kumar	INDIAN	Dabur Research Foundation, 22, Site-IV, Sahibabad, Ghaziabad – 201 010, Uttar Pradesh, India
[iii] JAGGI, Manu	INDIAN	Dabur Research Foundation, 22, Site-IV, Sahibabad, Ghaziabad – 201 010, Uttar Pradesh, India
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[vi] SANNA, Vinod	INDIAN	Dabur Research Foundation, 22, Site-IV, Sahibabad, Ghaziabad – 201 010, Uttar Pradesh, India
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[viii] DATTA, Kakali	INDIAN	Dabur Research Foundation, 22, Site-IV, Sahibabad, Ghaziabad – 201 010, Uttar Pradesh, India
[ix] MUKHERJEE, Rama	INDIAN	Dabur Research Foundation, 22, Site-IV, Sahibabad, Ghaziabad – 201 010, Uttar Pradesh, India
[x] BURMAN, Anand C.	INDIAN	Dabur Research Foundation, 22, Site-IV, Sahibabad, Ghaziabad – 201 010, Uttar Pradesh, India

3. TITLE OF THE INVENTION

"NOVEL 1,8-NAPHTHYRIDINE DERIVATIVES AS ANTICANCER AGENTS"

4. ADDRESS FOR CORRESPONDENCE OF APPLICANT/AUTHORISED PATENT AGENT IN INDIA	Telephone No.:
SUBRAMANIAM, NATARAJ & ASSOCIATES Attorneys - at - law Patent & Trademark Attorneys E - 556, Greater Kailash - II New Delhi 110 048	(+91) (11) 29210792; 29215603; 29216025 Fax No.: (+91) (11) 29226005; 29226012 Email: sna@vsnl.com

5. PRIORITY PARTICULARS OF CONVENTION APPLICATION

Country	Application No.	Filing Date	Name of Applicant	Title of the Invention
		NIL		

6. PARTICULARS FOR FIILING PCT NATIONAL PHASE APPLICATION

Country	Application No.	Filing Date	Name of Applicant	Title of the Invention
		NIL		

7. DECLARATIONS

(i) **Declaration by the inventors:**

We, the above named inventors are the true and first inventors for this invention and declare that the applicants herein are our assignees.

(a) Date: 28 November 2005

(b) Signature: S.K Agarwal
(c) Name: **AGARWAL, Shiv Kumar**

(b) Signature: Sanjay Kumar Srivastava.
(c) Name: **SRIVASTAVA, Sanjay Kumar**

(b)Signature: Maggi
(c) Name: **JAGGI, Manu**

(b) Signature: AP
(c) Name: **SINGH, Anu, T.**

(b) Signature: Vivek
(c) Name: **KUMAR, Vivek**

(b) Signature: S. Vinod Kumar
(c) Name: **SANNA, Vinod**

(b) Signature:
(c) Name: **SINGH, Gurvinder**

(b) Signature: Kakali Datta
(c) Name: **DATTA, Kakali**

(b) Signature:
(c) Name: **MUKHERJEE, Rama**

(b) Signature:
(c) Name: **BURMAN, Anand, C.**

We, the applicants hereby declares that:

- ☐ We are in possession of the above-mentioned invention.
- ☐ The provisional specification relating to the invention is filed with this application.
- ☐ There is no lawful ground of objection to the grant of a patent to us.
- ☐ We are the assignees of the true and first inventors.

8. Following are the attachments with the application:

(a) Provisional specification

(b) Statement and undertaking on Form 3

Fee Rs. .....4000.00..... in cash/Cheque/Bank Draft bearing No.....
Date .....on .....Bank

We hereby declare that to the best of our knowledge, information and belief the facts and matters sta herein are correct and we request that a patent be granted to us for the said invention.

H.Subramaniam
of SUBRAMANIAM, NATARAJ & ASSOCIATES
Attorney for the Application

To
The Controller of Patents
The Patent Office
At New Delhi

FORM 2

THE PATENTS ACT, 1970
(39 of 1970)
&
The Patent Rules, 2003

PROVISIONAL SPECIFICATION
(See section 10 and rule 13)

TITLE OF THE INVENTION

"NOVEL 1,8-NAPHTHYRIDINE DERIVATIVES AS ANTICANCER AGENTS"

We, **DABUR PHARMA LIMITED,** 3, Factory Road, Adjacent Safdarjung Road, New Delhi – 110 029, India.

The following specification describes the invention: